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Radiohalogenated Pyrimidines for Tumor Evaluation

by

(C) Yip Wan Lee

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Abstract

[^{82}Br]-6-Bromouracil ([^{82}Br]-6-BrU) was synthesized using a bromine for iodine exchange reaction. 2,2'-anhydrouridine reacted with $^{82}Br^-$ to afford [^{82}Br]-2'-bromo-2'-deoxyuridine ([^{82}Br]-2'-BrUdR). [^{34m}Cl]-1-(3'-Chloro-3'-deoxy-β-D-arabinofuranosyl)uracil ([^{34m}Cl]-3'-ClUdR) and [^{82}Br]-1-(3'-bromo-3'-deoxy-β-D-arabinofuranosyl)uracil ([^{82}Br]-3'-BrUdR) were synthesized by the reaction of halides with the 2',3'-lyxoepoxide of uridine. Radioactive bromo compounds were also prepared by direct thermal neutron activation of the corresponding non-radioactive brominated pyrimidines. The synthetic methods were generally preferred since higher radiochemical yields were obtained. One exception was [^{82}Br]-2'-BrUdR which was produced with equal efficiency by direct neutron activation.

Preferential tissue uptake of [^{82}Br]-6-BrU, [^{82}Br]-2'-BrUdR, [^{34m}Cl]-3'-ClUdR, [^{82}Br]-3'-BrUdR and [^{34m}Cl]-6-chlorouracil ([^{34m}Cl]-6-ClU) was evaluated using BDF$_1$ mice bearing Lewis Lung carcinomas. No selective uptake was observed for the compounds by any tissues. All compounds underwent rapid renal excretion. Dehalogenation was observed and was most extensive for [^{82}Br]-2'-BrUdR accounting for 46.73% of the injected dose. [^{34m}Cl]-3'-ClUdR was the most stable with only 5.65% dehalogenation. A small but definite biliary excretion pattern was also evident.

Chloro and bromo substituents in the compounds tested were not biosteres for carboxyl, hydrogen and hydroxyl

functions in the animal model tested.

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List of Abbreviations

Ac	Acetyl
Amax	Maximum radioactivity
Ara-C	1-(β-D-arabinofuranosyl)cytosine
Ara-FC	1-(β-D-arabinofuranosyl)-5-fluoro-cytosine
Ara-U	1-(β-D-arabinofuranosyl)uracil
Bz	Benzoyl
CDP	Cytidine-5'-diphosphate
CdR	2'-Deoxycytidine
CIMS	Chemical ionization mass spectrometry
CMP	Cytidine-5'-monophosphate (Cytidylate)
CR	Cytidine
CTP	Cytidine-5'-triphosphate
dCDP	2'-Deoxycytidine-5'-diphosphate
dCMP	2'-Deoxycytidine-5'-monophosphate
dCTP	2'-Deoxycytidine-5'-triphosphate
DNA	Deoxyribonucleic acid
DMF	Dimethylformamide
dTDP	Thymidine-5'-diphosphate
dTMP	Thymidine-5'-monophosphate
dTTP	Thymidine-5'-triphosphate
dUDP	2'-Deoxyuridine-5'-diphosphate
dUMP	2'-Deoxyuridine-5'-monophosphate
dUTP	2'-Deoxyuridine-5'-triphosphate
EC	Orbital electron capture
EIMS	Electron impact mass spectrometry
EOB	End of bombardment
Et	Ethyl
GIT	Gastrointestinal tract
'H-NMR	Proton nuclear magnetic resonance
HOAc	Acetic acid
HPLC	High pressure liquid chromatography
i	Iso
IT	Isomeric transition
m	(In IR) Medium (In 'H-NMR) Multiplets
Me	Methyl
Ms	Methylsulfonyl
MS	Mass spectrometry
MsCl	Methylsulfonyl chloride
MTS	Michigan Terminal System
n	(In nuclear reactions) Neutron (In statistics) Number of entries
n	(In chemial nonmenclature) Normal
NaOBz	Sodium benzoate
Ph	Phenyl
PRPP	5-Phosphoribosyl-1-pyrophosphate
RNA	Ribonucleic acid

S.D.	Standard deviation
t	Tertiary
T	Thymine
TdR	Thymidine
Tr	Triphenylmethyl
Ts	*p*-Toluenesulfonyl
U	Uracil
UASR	University of Alberta SLOWPOKE Reactor
UDP	Uridine-5'-diphosphate
UdR	2'-Deoxyuridine
UMP	Uridine-5'-monophosphate
UR	Uridine
UTP	Uridine-5'-triphosphate
UV	Ultraviolet
2'-BrUdR	2'-Bromo-2'-deoxyuridine
2'-IUdR	2'-Iodo-2'-deoxyuridine
3'-BrUdR	1-(3'-Bromo-3'-deoxy-β-D-arabinofuranosyl)uracil
3'-ClUdR	1-(3'-Chloro-3'-deoxy-β-D-arabinofuranosyl)uracil
5-FU	5-Fluorouracil
5-FUdR	5-Fluorodeoxyuridine
5-IUdR	5-Iododeoxyuridine
6-BrU	6-Bromouracil
6-ClU	6-Chlorouracil
6-FU	6-Fluorouracil
6-IU	6-Iodouracil

1. Introduction

In 1895 Röntgen discovered X-rays[1] and in 1898 the Curies discovered radium.[2] The first reported use of a radioisotope in humans was reported by Blumgart and Yens who administered a solution of radon gas in saline to human subjects.[3] Thus the era of nuclear medicine began.

The rapid growth of nuclear medicine as a science began soon after the introduction of cyclotrons in the nineteen-thirties and the development of nuclear reactors in the forties. Radionuclides of various elements were made available in sufficient quantities for the first time to the scientific community.

Since then, nuclear medicine has become an important and integral part of clinical and diagnostic medicine due primarily to the technological advancement in nuclear instrumentation, especially in the field of radiation detection and computation, and the availability of new radiopharmaceuticals in the past few decades.

Radiopharmaceuticals can be broadly defined as pharmaceuticals containing a radionuclide. Some are useful as therapeutic agents such as phosphorus-32, cobalt-60, iodine-131 and gold-198. Most radiopharmaceuticals are, however, employed as diagnostic or scanning agents whereby their radioactive properties are utilized to locate areas of interest or to provide physiological and biochemical information.

Early radiopharmaceuticals were primarily inorganic salts. An example is [^{131}I]-sodium iodide which was used for visualization of the thyroid gland. Chelates, inorganic complexes and organic compounds were used as the science of nuclear medicine developed. One of the major advances in nuclear medicine originated with the development of the molybdenum-99/technetium-99*m* type generators to which the popularization of nuclear medicine is much indebted. With the development of better designed and more economical reactors and accelerators, major medical research centers have embarked on ambitious research programs often using radioiosotopes previously unavailable.

Most radiopharmaceuticals are used in diagnostic procedures. A diagnostic agent of general applicability to detect most types of tumors would have significant clinical implications. An early diagnosis is often equated with a good prognosis.

Design of organ-imaging radiopharmaceuticals has been reviewed by Counsell and Ice.[4] Design concepts are classified artificially and often subjectively. Concepts sometimes overlap and some radiopharmaceuticals are products of more than one approach.

1. Availability of radionuclides

The role of iodide in thyroid iodine metabolism and phosphorus in hematopoiesis was established prior to availability of radioactive iodine and phosphorus. When ^{128}I and ^{32}P were made available these radionuclides

were utilized immediately in nuclear medicine. The usefulness of some radiopharmaceuticals can also be improved by the use of a different radionuclide of the same element. An example is the replacement of ^{131}I by ^{123}I.

2. Nuclear and chemical properties

The nuclear properties of radionuclides (physical half-lives and emission characteristics) determine their suitability for use in nuclear medicine. Some radionuclides e.g. ^{111}In, ^{113m}In and ^{99m}Tc have been described as "ideal" medical nuclides.

The chemical reactivity of radionuclides determines their ease of incorporation into pharmaceuticals. The usefulness of radionuclides is sometimes compromised by their chemical innertness and/or lack of useful synthetic procedures.

3. Pharmacology

Many imaging agents were products of pharmacological research, for example diuretics (^{197}Hg-chlormerodrin) and antibiotics (radiolabelled bleomycins).

4. Biochemistry

The biochemical concept is best exemplified by iodine metabolism by the thyroid gland (radioactive iodide), protein metabolism ([^{75}Se]-L-selenomethionine), steroid metabolism ([^{131}I]-19-iodocholesterol), glucose metabolism ([^{18}F]-2-deoxy-fluoro-D-glucose) and nucleic acids metabolism ([^{131}I]-5-iodo-2'-deoxyuridine,

5-IUdR).

5. Mechanism of localization

Mechanisms of localization provide a basis of design for many radiopharmaceuticals. Examples include the swamp effect (^{99m}Tc-pertechnetate as bone imaging agent), diffusion (^{99m}Tc-pyrophosphate in bone imaging and radiolabelled fatty acids in myocardial studies), ion exchange (Ga^{3+}), extraction by hepatic parenchymal polygonal cells (^{131}I-Rose Bengal), phagocytic action of hepatic Kupffer cells (^{198}Au-colloidal gold), pH shift ([^{123}I]-iodo-*i*-propylamphetamine), fibrin formation (^{131}I-fibrinogen), and antibody-antigen reaction (radiolabelled antibody to carcinoembryonic antigen, CEA).

6. Liposomes and lipoproteins as carriers for radiotracers

Successful application of liposomes and lipoproteins as radiotracer carriers to enhance target specificity have been reported in the literature e.g. unilamellar vesicles-^{99m}Tc-Sn-DTPA).

It was the intent of this research project to evaluate certain uracil derivatives for use in diagnostic oncology; not particularly as localizing agents, but as indicators of the dynamic state of the tumor mass before and after treatment, thereby providing a true and early indication of therapeutic efficacy.

Historically the use of 5-IUdR as a tumor localizing agent was first suggested by Welch and Prusoff in 1960.[9] Since then, numerous purine and pyrimidine analogues have

been investigated as potential scanning agents.[4-11] The accumulation of activity could serve as an indication of cell proliferation rate while the loss of activity from the tumor mass would suggest cell lysis.[9]

Biological studies using 5-IUdR have indicated significant uptake in organs of high mitotic index, such as the intestinal epithelium, bone marrow and the tumor. The loss of the halogen label from the molecule was also rapid and has been associated with a reduction of the 5,6 double bond of the pyrimidine base unit.[12] This resulted in high blood activity[7,8] as early as fifteen minutes post injection.

2. Literature Survey

2.1 Pyrimidine Metabolism

Specificity for many disease causing organisms is not a major problem in the design of conventional pharmaceuticals. The causative agents differ biochemically and/or histochemically from the host. Such differences are often exploited in devising new drugs. One example is folic acid which is an essential growth factor for both bacteria and animals. Folic acid is synthesized from *p*-aminobenzoic acid by bacteria which cannot utilize exogenous folic acid. However, the situation is distinctly different for animals which must rely upon a dietary supply of the vitamin. This biochemical difference provides the basis for the use of sulfanilamide, an anti-metabolite and structural analogue of *p*-aminobenzoic acid.

The causes of most types of cancers are unknown, although many agents have been shown to be carcinogenic in nature. Cancer is a general term used to describe an uncontrolled proliferation state of certain cells. Cancerous cells are similar to the host's cells with respect to many physical and biochemical parameters. Some differences, mostly quantitative, have been reported:[4,13,14]

1. Lower pH
2. Elevated free radical concentration
3. Increased antigen and hormone peptide content
4. Higher nucleic acid, protein and polysaccharide meta-

bolic rates

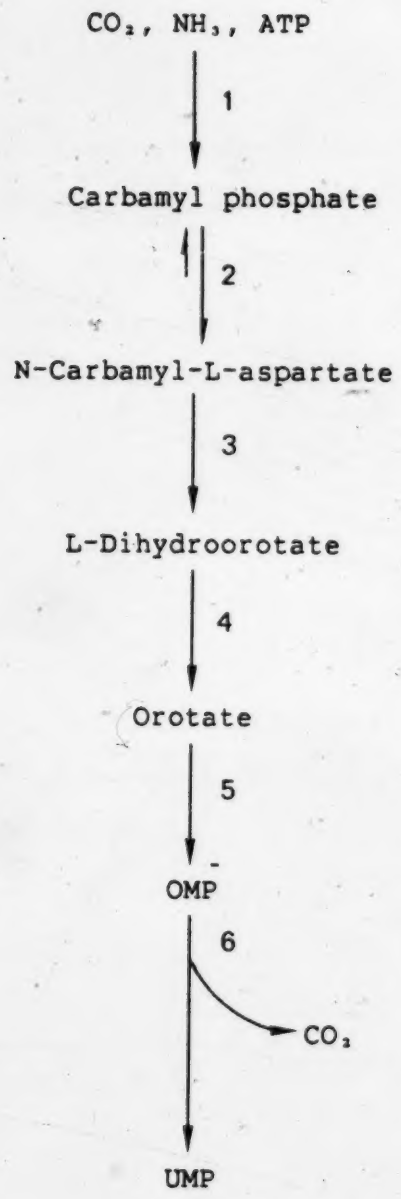
5. Increased requirement for exogenous zinc
6. Increased vasculature distended with increased blood volume in and around the tumor
7. Increased tumor membrane permeability
8. Increased extracellular space
9. Edema in and around the tumor
10. Changes in the sodium, potassium and calcium ions concentrations.

Pyrimidines are physiological molecules that are basic components of co-enzymes, ribonucleic acids (RNA) and deoxyribonucleic acids (DNA). They are involved in most aspects of cellular activities. It is this obvious significance to cellular functions that have made them prime candidates for chemotherapeutic and scanning agents.

Many reviews dealing with pyrimidine intermediary metabolism have appeared.[8-11] A summary of the pathways is presented in the following pages. The sequence of intracellular events leading to the formation of uridylate is known as the *de novo* pathway of pyrimidine biosynthesis. A schematic representation of the pathway is shown in Scheme 1.1. The trivial names of the enzymes are listed in Table 1.1. The pathway is universal in occurrence. Most animals do not have a dietary need for pyrimidines which are synthesized *in vivo* from simple organic molecules. Carbon dioxide, glutamine or ammonia and aspartate react to form carbamyl aspartate in a two-step reaction, carbamyl phosphate being

Table 1.1: Enzymes of the pyrimidine *de novo* pathway of biosynthesis.

Enzyme	Trivial names
1	Carbamyl phosphate synthetase
2	Aspartate carbamyltransferase
3	Dihydroorotase
4	Dihydroorotate dehydrogenase
5	Orotate phosphoribosyl transferase
6	Orotidine-5'-phosphate decarboxylase



Scheme 1.1: The *de novo* biosynthesis of UMP.

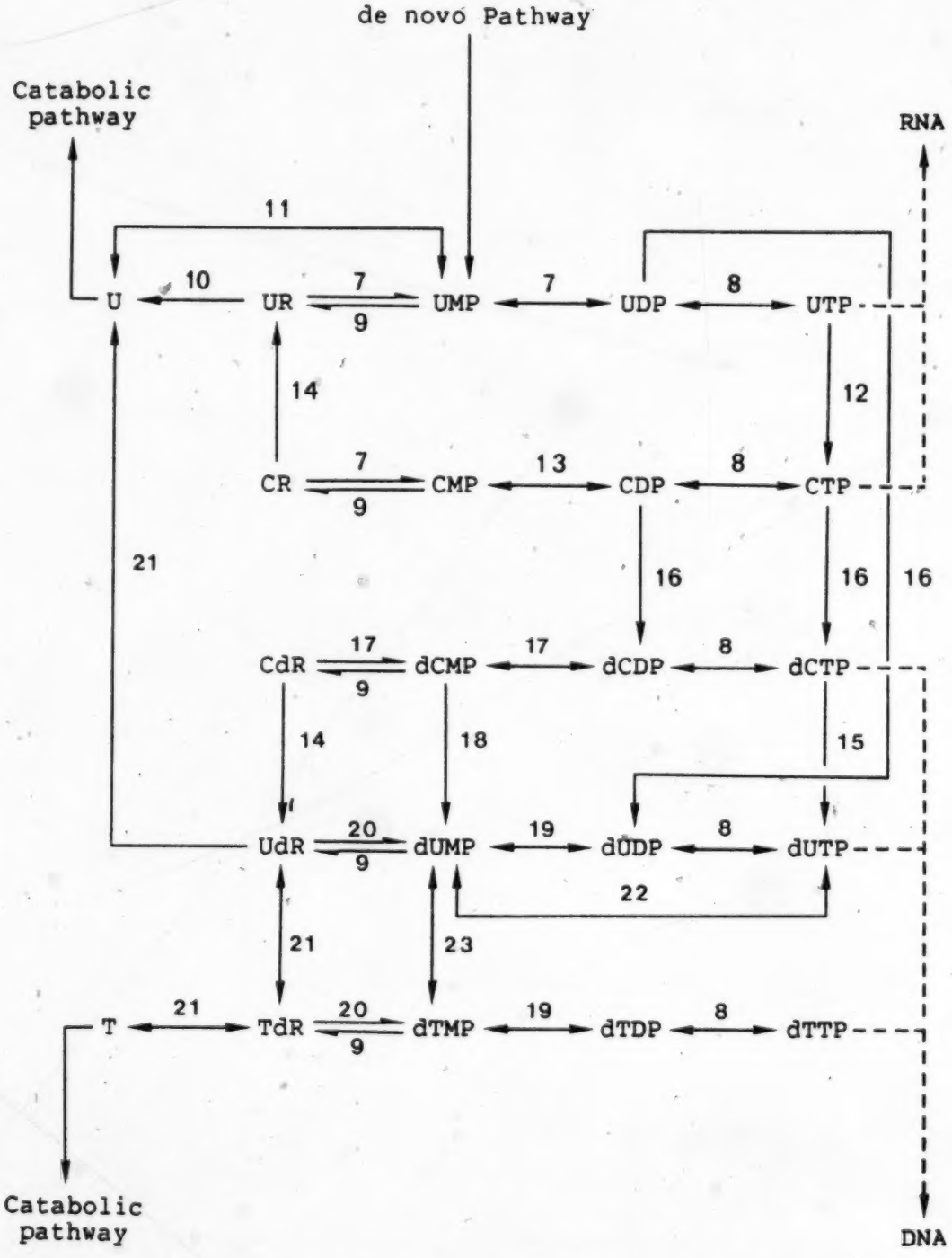
the intermediate. The aspartate undergoes intramolecular cyclization to dihydroorotate which is subsequently reduced by dihydroorotate dehydrogenase to orotate. Coupling between N-1 of the orotate and C-1 of PP-ribose-P affords orotidylate or OMP which is quickly decarboxylated to uridylate or UMP. Uridylate is the first essential pyrimidine nucleotide formed in the *de novo* pathway and is the precursor to other pyrimidines commonly found in nucleic acids.

In addition to the *de novo* pathway, a "salvage" pathway exists in most eukaryotic organisms for re-utilization of preformed pyrimidines arising from metabolic activities or from an exogenous source. These alternate pathways are depicted in Scheme 1.2. with the corresponding metabolic enzymes listed in Table 1.2.

UMP arising from the *de novo* pathway can be converted to the di- and tri-phosphates by kinases. UMP can also be degraded to uracil by way of uridine. The free base, uracil, can re-enter the nucleotide pool *via* two processes. Uracil phosphoryltransferase can transform uracil and 5-phosphoribosyl-1-pyrophosphate (PRPP) into UMP *via* a reversible reaction in which the equilibrium lies in the direction of degradation. The enzyme will also accept 5-FU as an alternate substrate. The same enzymatic activity has been found lacking in 5-FU resistant mouse leukemia L1210 cells.[20] Similar enzymatic reactions have been observed for cytosine and thymine. Uridylate is also formed from uracil in a step-wise process *via* uridine by the sequential action of a

Table 1.2: Enzymes of the pyrimidine "salvage" pathways and interconversion of pyrimidine nucleotides.

Enzyme	Trivial name
7	Uridylate-cytidylate kinase
8	Nucleoside diphosphate kinase
9	5'-Nucleotidase
10	Uridine phosphorylase
11	Uracil phosphoribosyltransferase
12	CTP-synthetase
13	Cytidylate kinase
14	Cytidine deaminase
15	Deoxycytidine triphosphate deaminase
16	Ribonucleotide reductase
17	Deoxycytidine kinase
18	Deoxymonophosphate deaminase
19	Thymidylate kinase
20	Thymidine kinase
21	Thymidine-deoxyuridine phosphorylase
22	dUTP pyrophosphatase
23	Thymidylate synthetase



Scheme 1.2: "Salvage" pathways and interconversion of pyrimidine nucleotides.

phosphorylase and kinase. The action of the latter is inhibited by its own end product, UTP. Uridine phosphorylase catalyzes the reversible transition between uracil and uridine. Uridine is the preferred substrate; deoxyuridine, thymidine, 5-FUR and 5-FUdR are less active substrates.

Cytidylate is formed from the amination of UTP in the presence of glutamine or ammonia and CTP synthetase. Deamination occurs only at the nucleoside level. Both cytidine and deoxycytidine are substrates for cytidine deaminase, as are their 5-halo derivatives. The glycosidic bond of cytidine and deoxycytidine, unlike uridine, is not cleaved by phosphorylases in most animals. Deamination to uridine or deoxyuridine must precede the glycosidic cleavage. Formation of CMP is catalyzed by uridylate-cytidylate kinase, an enzyme that is regulated by one of its end products, CTP. The ultimate anabolic fate of UTP and CTP is incorporation into RNA.

A minor constituent of the acid-soluble fraction of the cell is deoxyribonucleotides, of which deoxycytidine phosphate is the most abundant. Deoxyribonucleotides are formed biosynthetically, from anabolic breakdown of endogenous deoxyribonucleotides and also arise from dietary intake.

Enzymatic reduction of ribonucleotides is the primary source of deoxyribonucleotides in nature. In animal cells, the reductive process occurs at the diphosphate level and requires the enzyme nucleotide reductase and a hydrogen-carrier system, thioredoxin. The reduction step does not

involve the glycosidic bond and the resultant deoxyribonucleotide retains the configuration of the precursor ribonucleotide, including the 2'-hydrogen atom *cis* to the N-glycosidic bond. Deoxycytidine is phosphorylated to its 5'-monophosphate by deoxycytidine kinase with deoxycytidine as the preferred substrate. Cytidine, uridine and thymidine are alternate substrates.

Deoxyuridine and its nucleotides are derived from cytidine and dCMP by the action of deaminase and from the reduction of UDP by ribonucleotide reductase, which also reduces CDP to dCDP. A non-reversible dephosphorylation reaction converts dUTP to dUMP. Phosphorolysis of deoxyuridine produces uracil which is also the final catabolic product of cytidine and deoxycytidine.

The methylation of dUMP by thymidylate synthetase provides the main source of thymidylate in most living organisms. This methylation reaction has been studied in detail.[21-24] The one-carbon unit donor has been identified as 5,10-methylene H_4-folate which is present in a catalytic quantity. Inhibition of thymidylate synthetase is believed to be the mechanism of the toxic action of 5-FU and 5-FUdR, which cannot be phosphorylated beyond their monophosphate level.[25]

Thymine, as the free base, is poorly utilized by most living organisms. Some living cells can utilize dietary thymidine, which is phosphorylated by thymidine kinase before entering the nucleotide pool. The activity of thymidine

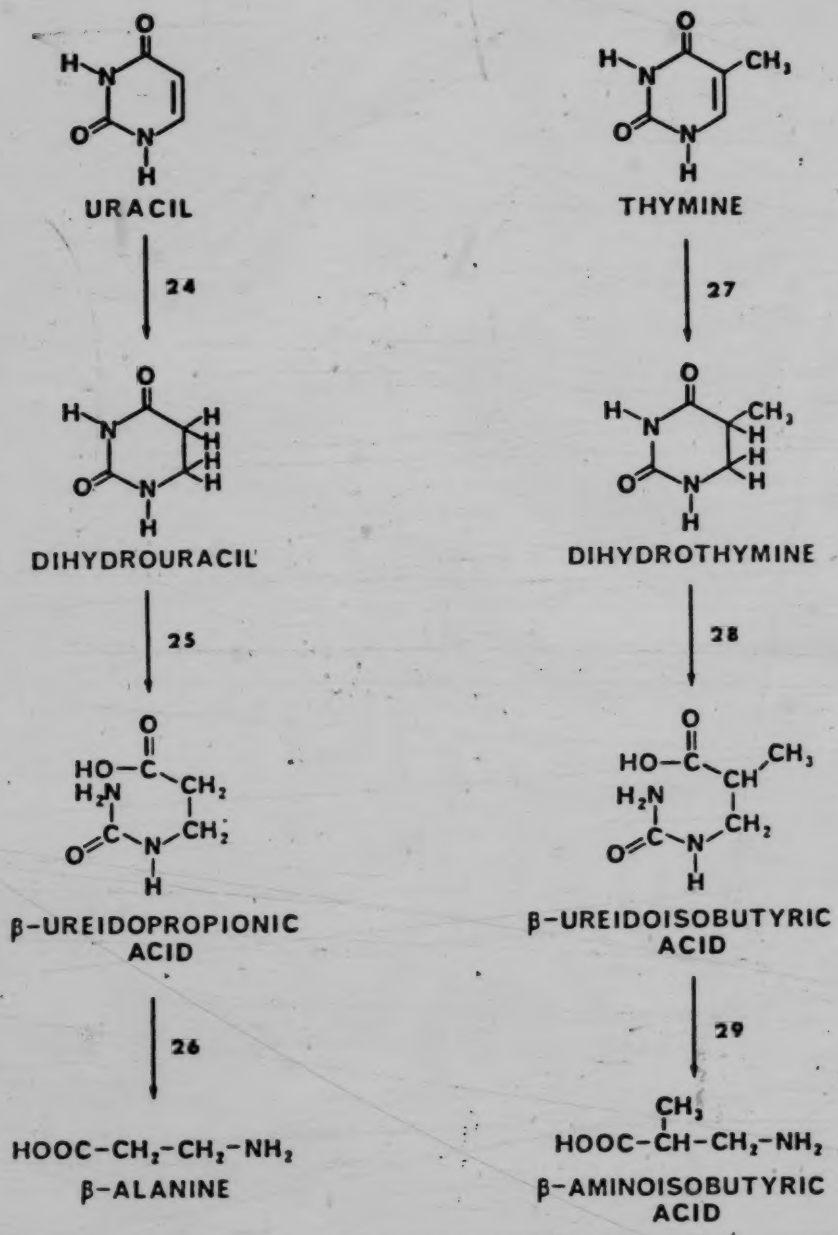
kinase has been correlated with the mitotic activity of its host cells and is inhibited by dTTP.[24,27]

Deoxyribonucleotides are incorporated into DNA at the triphosphate level. Deoxycytidine and deoxyuridine are anabolised to uracil which can undergo further anabolic reactions, or can re-enter the pyrimidine salvage pathway. Similar metabolic routes are followed by thymine.

Nucleic acids are degraded by ribonucleases and deoxyribonucleases. The released ribonucleotides are catabolised by a series of enzyme mediated reactions which may include dephosphorylation, deamination and glycosidic bond cleavage. The catabolism of the pyrimidine bases and the interacting enzymes are represented in Scheme 1.3 and Table 1.3 respectively. Degradation occurs largely in the adult liver. Reduction of the 5,6 double bond of uracil and thymine is perceived to be the initiating step. The 5,6-dihydro derivatives are oxidatively cleaved at the 3,4 position forming β-ureidopropionate and β-ureidoisobutyrate respectively. Enzymatic deamination and decarboxylation yield the corresponding β-amino acids. Virtually all pyrimidines follow a similar catabolic pathway and only trace quantities are excreted unchanged, primarily as orotic acid and orotidine.

Table 1.3: Catabolic enzymes of pyrimidine metabolism.

Enzyme	Trivial name
24	Dihydrouracil dehydrogenase
25	Dihydrouracil hydrase
26	β-Ureidopropionic acid decarboxylase
27	Dihydrothymine dehydrogenase
28	Dihydrothymine hydrase
29	β-Ureidoisobutyric acid decarboxylase



Scheme 1.3: Catabolism of pyrimidine bases.

2.2 Analogues of Pyrimidines

Nucleotides, nucleosides and their bases are of universal occurrence. They are found in all living cells. They are responsible for many life functions and are themselves the building block of life. Many are useful chemotherapeutic agents. They affect not only the biosynthesis of nucleic acids, but also that of carbohydrates, proteins and other cell constituents. They have the potential to undergo numerous biotransformations *via* numerous metabolic routes. The diversity of their functions is self evident as illustrated by Scheme 1.1 and Scheme 1.2 which represent only a small number of the overall metabolic pathways.

Since the proposal of the term "nucleosides" by Levene and Jacobs in 1909,[28] the volume of literature on the topic has increased tremendously. Many review articles and books have beeen published on the subject.[18,28-34] The interest in the medical application of these compounds has spurred the isolation of numerous naturally occurring nucleosides and the syntheses of many novel ones. In the process, the heterocycle as well as the carbohydrate moiety have been modified in an attempt to custom design biological activities. Modifications usually involve one or more of the following:

1. Modifications of the heterocycle
2. Changes of the substituent groups attached to the heterocycle
3. Structural modifications within the carbohydrate moiety

4. Replacement of substituent groups on the carbohydrate ring.

In view of the fact that a very vast number of analogues have been reported only those with modifications at positions C-6 of uracil and C-2' and C-3' of pyrimidines and purines will be reviewed. The numbering system used for uridine is represented by numbers in the heterocyclic ring which is also known as the aglycone and by primed numbers for the furanose ring (Fig. 1.1). Nucleosides can adopt two principal conformations with respect to the C-1'-N-1 bond.[30] The O^2 atom of the aglycone is situated over the furanose ring in the *syn* conformation and points away from the ring in the *anti* mode which is the preferred conformation of most ribo- and deoxyribonucleosides.[31]

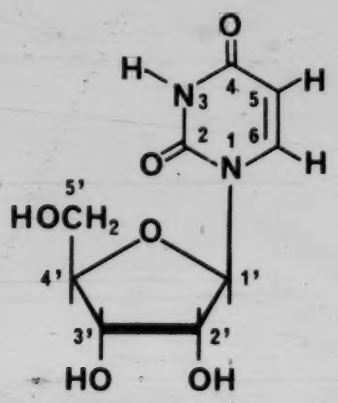


Fig. 1.1: Uridine.

The five-member ring of furanose is not planar but puckers (pseudorotates) in a twist, T (half chair) or an envelope, E, form. The term C-2'-*endo* means that the C-2' atom is out of the plane of the other four atoms, C-1', C-3', C-4' and O-1', by about 0.5 Å and on the same side as C-5'. In C-2'-*exo*, the C-2' atom is on the opposite side of the plane with respect to C-5'.[30]

The energy barrier between the various puckering modes is believed to be low, about 5 Kcal M^{-1}. Steric and electronic properties of substituents on the sugar ring have been qualitatively correlated with the different puckering modes which in turn affect the orientation of the glycosidic (C-1'–N-1 and C-4'–C-5') bond.[30,34]

The furanose ring carries a substituent at each of the C-2' and C-3' position allowing a total of four possible configurational isomers, *ribo*, *arabino*, *xylo and lyxo*, as illustrated by Fig. 1.2.

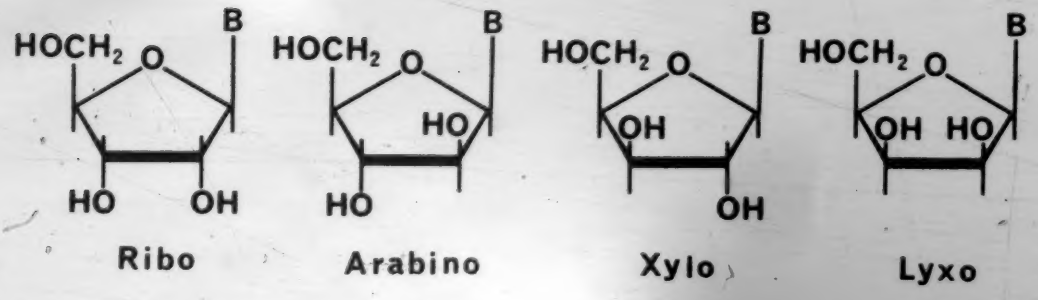


Fig. 1.2: Configurational isomers of furanose rings.

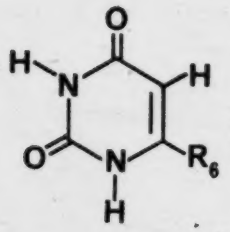
2.2.1 Analogues of 6-Substituted Uracils

A partial compilation of 6-substituted uracils, uridines and cytosines is given in Table 2.1 to Table 2.8. Many functionalities have been introduced into the C-6 position. Most analogues are designed to interact with orotidine decarboxylase,[48,51,52] orotidine phosphorylase,[48,53] thymidine phosphorylase[40-42,45,46] or some other enzyme systems.[43,44]

Table 2.1 is a list of some 6-monosubstitued uracils. 6-Alkyl and 6-aryl substituted uracils (group 1b) did not show any reproducible biological activity against sarcoma-180, carcinoma-755 and leukemia-1210 *in vivo*.[39]

Baker *et al* using the enzymes thymidine phosphorylase[40-42,46,47] and cytosine nucleoside deaminase[44] concluded that 6-alkyl, 6-aryl and 6-arylalkyl substituents contributed significantly to the hydrophobic bonding between the enzyme and substrate.[40-42,44,47] The same authors, in a series of articles, systematically investigated the structural requirements of uracils for inhibition of thymidine phosphorylase.[40-42,45,46] They demonstrated that hydrophobic bonding of uracil to the enzyme was a pre-requisite to biological action. An *n*-butyl substituent at C-1 was observed to confer a nine-fold increase in bonding capability to uracil when compared to a C-1 methyl group. A similar hydrophobic bond enchancement of biological activity up to 150 times was also observed for 6-benzyl-5-bromouracil relative to uracil. Neither the 2-oxo nor 4-oxo was reported

Table 2.1: 6-Substituted uracils.



(1)

	R_6	Reference
a.	$-NH_2$	37, 38
b.	-alkyl; -aryl; -arylakyl	37-47
c.	-NHR	44-48
d.	$-CH_2F$	49
e.	$-CF_3$	49, 50
f.	-NHOH	51
g.	$-NHCH_3$; -OH	52
h.	-SH; $-SCH_3$	53
i.	$-SO_3H$; $-SO_2CH_3$; $-SO_2NH_2$	54, 55
j.	-F	52
k.	-Cl	52, 53, 56, 57
l.	-Br	56, 57
m.	-I	56-58

to be necessary for the formation of the enzyme-substrate complex. It was suggested that the 1-H and 3-H were bonded to the enzyme and that the bonding power of 1-H was strongly dependent upon its own acidity. An increase in the acidity of the 1-H due to an electron withdrawing group at C-5 or C-6 position strongly increased the binding ability of 1-H. The effect exerted by a C-5 substituent was greater than that of a C-6 as an electron withdrawing group located at C-6 would interfere with the hydrophobic bonding of the molecule to the enzyme.

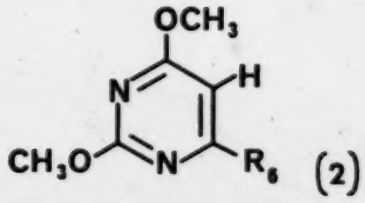
6-Aminouracils (group 1c) have also received considerable interest.[44-48] 6-Anilinouracil has been shown to be a potent inhibitor of DNA polymerase III from *B. subtilis*.[48] Other 6-anilinouracils have been reported to be effective inhibitors of thymidine phosphorylase.[46,47] The presence of a phenyl ring appears to be responsible for the hydrophobic bonding between the enzyme and the substrate[46,48] and uracils substituted at C-6 with $-NH_2$ and $-N_2OH$ without a non-polar component have been found to be ineffective against *E. coli*.[33]

Among the compounds of group 1i, the sulfonic acid ($R_6=SO_3H$) analogue was found to inhibit utilization of orotidine by orotidine dependent bacteria. 6-Methanesulfonyluracil ($R_6=SO_2CH_3$) and uracil sulfonic acid competitively inhibited OMP decarboxylase[32,46] and the growth of *L. bulgaricus* in the presence of orotidine.[46] 6-Methanesulfonyluracil was also found effective in inhibiting L1210,

L4946, L5178 lymphomas and sarcoma-180.[48] Greenbaum reported that the 6-sulfonamidouracil possessed the same biological activity as the methanesulfonyl analogue.[54]

Studies involving 6-halogenouracils (group 1j to m) have shown that 6-FU is completely inactive against 14 microorganisms of which 9 were inhibited by 5-FU.[49] This inactivity has been attributed to its susceptibility to both acid and base catalyzed hydrolysis.[49,55] 6-ClU showed limited biological activity while its 2,4-dimethoxy derivatives (Table 2.2) were considerably more active.[50]

Table 2.2: 6-Substituted 2,4-dimethoxyuracils.



(2)

	R_6	Reference
a.	-CN; $-N_3$; $-N(CH_2)_3Cl$; -F; -Cl; -Br; -I	53, 56, 58
b.	-SH; $-SCH_3$; $-SO_2CH_3$	53

Some of the reported 6-substituted-2,4-dimethoxyuracils are listed in Table 2.2. The 6-Cl, -SH, $-SCH_3$ and $-SO_2CH_3$ substituted 2,4-dimethoxyuracils have all been found to be inhibitory to *E. coli*.[53]

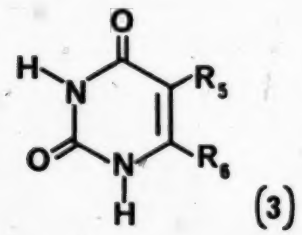
A partial list of the 5,6-disubstituted uracils reported to date are shown in Table 2.3. No biological activity has been reported for the majority of these analogues. The compounds shown in group **3c** having a C-5 chloro and a C-6 alkyl substituent have been tested against sarcoma-180, carcinoma-755 and leukemia-1210 with nonreproducible results.

Pfleiderer and Deiss synthesized a group of 5,6-dihalogenouracils which are shown in Table 2.4 for which no biological activity was reported.[57]

Chae *et al*[60] synthesized some 6-uridylylaldehydes (Table 2.5) and reported that **5a** (R_2 = SH) and 5b ($R_2 = C_6H_5$) inhibited the incorporation of orotidine into RNA and DNA. Other compounds listed showed either little or no activity.[60] Compounds **5d** were tested for antitumor activity but the results were nonreproducible.

A number of 2-amino-4,6-disubstituted pyrimidines were synthesized by Israel *et al* (Table 2.6).[61] Compounds **6a** (R_6 = OH), **6b** (R_6 = Cl) and **6c** (R_6 = SH) were found to be active against *S. faecalis*. It was also shown that **6a** and **6c** were moderately inhibitory to C1498 tumor cells and that **6a** was effective against P1534 lymphatic leukemia.

Table 2.3: 5,6-Disubstituted uracils.



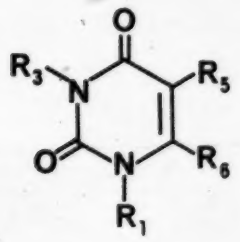
(3)

	R_5	R_6	Reference
a.	$-CH_3$	$-F$; $-Cl$; $-NCH_2C_6H_5$	59
b.	$-F$	$-CH_3$; $-CF_3$; $-C_2F_5$	49
c.	$-Cl$	$-CH_3$; $-C_2H_5$; $-Pr$; $-i$-Pr; $-n$-C_{16}; $-C_6H_5$; $-COOCH_3$	39
d.	$-Cl$	$-Cl$; $-Br$	57
e.	$-Br$	$-CH_3$	39
f.	$-Br$	$-Cl$; $-Br$; $-I$	57
g.	$-I$	$-Cl$; $-Br$; $-I$	57

Some 6-substituted cytosines have been reported in the literature (Table 2.7). Compound 7b ($R_6 = SCH_3$) and 7d ($R_6 = Cl$) exhibited moderate activity against C1498 leukemia.[63] The antifungal activity of 7c ($R_6 = F$) was described as limited.[52]

A number of 1,6-disubstituted uracils have been reported (Table 2.8). The compounds shown in group 8a ($R_5 = H$) were reported to be biologically inactive.[37,64,65] The ribose-5'-monophosphate analogues were degraded by a number

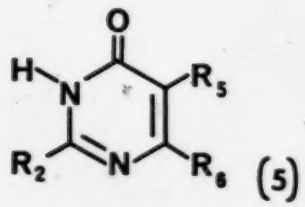
Table 2.4: 5,6-Dihalogenouracils.[87]



(4)

	R_1	R_3	R_5	R_6
a.	-H	$-CH_3$	-H	-Br
b.	-H	$-CH_3$	-H	-I
c.	-H	$-CH_3$	-Cl	-Cl
d.	-H	$-CH_3$	-Br	-Cl
e.	-H	$-CH_3$	-I	-Cl
f.	-H	$-CH_3$	-I	-I
g.	$-CH_3$	$-CH_3$	-H	-Br
h.	$-CH_3$	$-CH_3$	-H	-H
i.	$-CH_3$	$-CH_3$	-Cl	-Cl
j.	$-CH_3$	$-CH_3$	-Br	-Cl
k.	$-CH_3$	$-CH_3$	-I	-Cl
l.	$-CH_3$	$-CH_3$	-I	-Br
m.	$-CH_3$	$-CH_3$	-I	-I

Table 2.5: 2,6-Di- and 2,5,6-trisubstituted pyrimidines.



(5)

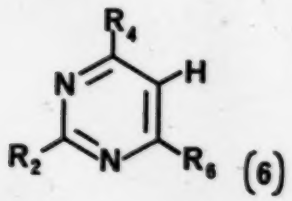
	R_2	R_5	R_6	Reference
a.	-OH; -SH	-F	-CHO	60
b.	$-C_6H_5$; -*p*-$C_6H_4C_6H_5$	-H	-CHO	60
C.	$-C_6H_5$; -*p*-C_6H_4F	$-CH_3$	-CHO	60
d.	-SH	-H	$-C_2H_5$; -*i*-Pr; -n-C_{16}; $-C_6H_5$; -COOH	39

of phosphatases.[38]

Von Janta-Lipinski and Langen[40] reported the synthesis of a series of 6-substituted thymidines (group 8b, $R_5 = CH_3$). The β-D-glucopyranosyl and β-D-ribofuranosyl nucleoside derivatives of 6-fluorothymine possessed antiviral properties. The activity of the latter was one order of magnitude less than that of the former but was comparable to that of the β-D-glucofuranosyl nucleoside derivative of 6-aminothymine.

Gut *et al* reported the syntheses of certain 6-substituted 2-thiouracils.[37] Compounds 9a and 9b were found to be inactive toward *E. coli*.

Table 2.6: 2,4,6-Trisubstituted pyrimidines.[11]



(6)

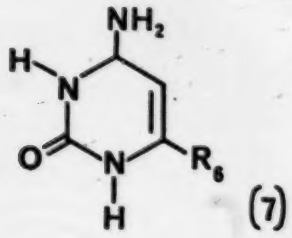
	R_2	R_4	R_6
a.	$-NH_2$	-OH	$-NH_2$; -OH
b.	$-NH_2$	-Cl	$-NH_2$; -Cl
c.	$-NH_2$	-SH; $-SO_2H$	$-NH_2$

The C-6 carbon has been replaced with nitrogen by Handschumacher to afford 6-azauridine (compound 10).[10] 6-Azauridine was effective against a broad spectrum of DNA and RNA viruses in high concentration.[11]

2.2.2 Analogues of 2'-Substituted-2'-deoxyuridines

Modification of the substituents on the furanose ring has commanded more attention than that of the heterocycle. The C-2' position is of great biological implication. The hydroxyl function at C-2' distinguishes RNA from DNA. Its presence bestows upon nucleosides many biochemical properties not readily explained by its steric and electronic properties. The chemistry of 2'-substituted nucleosides is

Table 2.7: 6-Substituted cytosines.



(7)

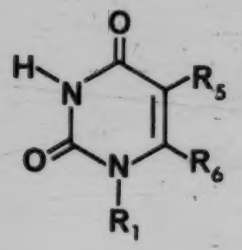
	R_6	Reference
a.	$-NH_2$	52
b.	$-SH$; $-SCH_3$	63
c.	$-F$	52, 62
d.	$-Cl$	62, 63

also synthetically more viable.

Many 2'-substituted nucleosides have been synthesized with substituents other than hydrogen and hydroxyl for use as probes to study the biochemical role of the C-2' function and as potential chemotherapeutic agents (Table 3.1).

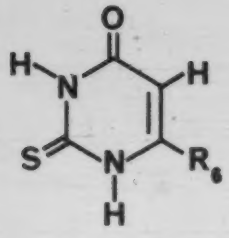
Glycosidic bonds of most 2'-halogeno-2'-deoxyuridines possess an *anti* conformation with C-2' endo.[30,78] 2'-Fluoro-2'-deoxyuridine 11a is unique in that it assumes a *syn* conformation with C-3' *endo* and C-4' *exo*.[30,73,103,104] In this respect the 2'-fluoro analogue resembles a ribose.

Table 2.8: 1,6-Disubstituted pyrimidines.

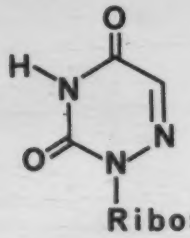


(8)

	R_1	R_6	Reference
a.	-ribose; -2'-deoxyribose; -ribose-5'-monophosphate; -ribose-5'-diphosphate	$-CH_3$	37, 64, 65
b.	-β-D-glucopyranosyl; -β-D-ribofuranosyl; -5'-deoxy-5'-fluoro-β-D-glucopyranosyl; -D-mannopyranosyl; -D-arabinopyranosyl	-F; $-NH_2$	66
c.	-5'-O-acetyl-2'-3'-O-*i*-propylidenofuranosyl	-CN; $-CONH_2$; $-CSNH_2$; $-CH_2NH_2$; $-CH_2OH$; $-CH_2Cl$; $-CH_2SC_2H_5$; $-CH_3$; $-CH_2CN$; $-CH_2COOH$; -tetrazole	67

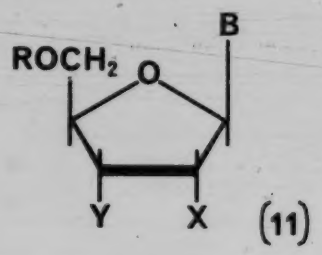


(9) a $R_6 = CH_3$; b $R_6 = NH_2$



Ribose (10)

Table 3.1: 2'-Substituted-2'-deoxyribofuranosides.



(11)

	X	Y	B	Reference
a.	-F	-OH	U	72-74
b.	-Cl	-OH	U	72, 73, 75-80
c.	-Br	-OH	U	72, 73, 80-82
d.	-I	-OH	U	73, 80, 83, 84
e.	-NHR	-OH	U	75, 85-88
f.	$-N_3$	-OH	U	85, 87, 89
g.	$-NHCOCF_3$	-OH	U	90
h.	-SH	-OH	U	91, 92
i.	-SAc	-OH	U	91
j.	-SH	$-NH_2$	U	93
k.	-F; -Cl; -Br	-OH	T	72
l.	-Br	-OH	5-BrU	81
m.	-F	-OH	5-FU	94
n.	$-NH_2$	-OH	C	77
o.	$-N_3$	-OH	C	95
p.	-SH	-OH	C	96
q.	-F	-OH	C	97

Table 3.1: 2'-Substituted-2'-deoxyribofuranosides (cont'd)

	X	Y	B	Reference
r.	-Cl	-OH	C	77, 78
s.	$-NH_2$	-OH	A	98, 99
t.	$-N_3$	-OH	A	99, 100
u.	-F; -Cl; -Br; -I	-OH	A	75, 101, 102
v.	$-NH_2$; $-N_3$	-OH	G	103
w.	-F	-OH	G	104

The 5'-monophosphate of **11a** has been reported to be a good substrate for *E. coli* thymidylate synthetase.[105] The diphosphate was polymerized by *E. coli* polynucleotide phosphorylase and transcription was observed to be 55% of that with UTP.[105] The difluoro analogue **11m** has also been demonstrated to be a good substrate for phosphorylase from Ehrlich ascites cells.[74]

Varying degrees of biological activity have been reported for 2'-chloro-2'-deoxyuridine **11b**. The 5'-di- and -triphosphates of **11b** have been reported to be good substrates for polynucleotide phosphorylase.[77,78] However, Armstrong and Eckstein[78] reported that 2'-ClUTP did not replicate well and suggested a slower reaction rate rather than a weak binding force to account for their observation.

Poly-2'-deoxyuridine and the 2'-fluoro, 2'-chloro and 2'-amino analogues did not form a helical structure whereas

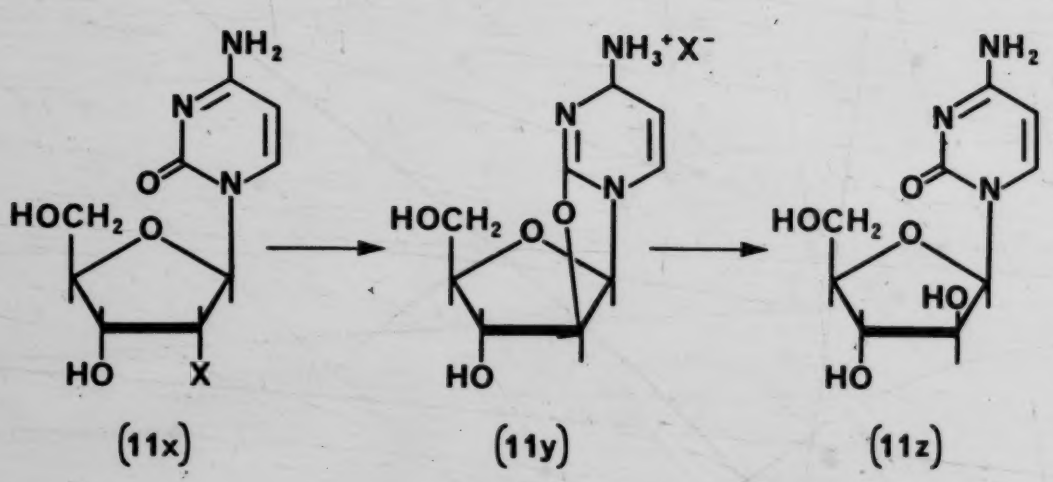
the 2'-O-methyl- and 2'-O-ethyl analogues did. The lack of helical stability has been attributed to the absence of the 2'-oxygen atom. It was not due to the absence of intramolecular hydrogen bonding[108] which is generally accepted as one of the stabilizing forces in substrate-enzyme complex formation.[30]

The first tissue distribution studies for 2'-bromo-11c[82] and 2'-iodo-2'-deoxyuridine 11d[83] were recently reported by our research group. No preferential tissue uptake has been noted for either compound.

It was reported that 2'-chlorocytidine 11r, 2'-chloro-5-fluorocytidine and 2'-fluorocytidine 11q exhibited some biological activity against leukemia P815 *in vitro* which was blocked by deoxycytidine but not by cytidine or thymidine.[107]

A hypothesis to explain the mode of action for 2'-halogenocytidine and deoxycytidine has been put forward (Scheme 2.1). 2'-Halogenocytidines 11x are metabolised to the biologically active Ara-C 11z *via* the intermediate 2,2'-anhydrocytidine 11y.[109]

Many analogues of 2'-amino, 2'-azido nucleosides have been prepared. 2'-Amino-2'-deoxyuridine 11e and its aminoacyl derivatives have been reported to be weakly active against *E. coli*.[29,110,111,112] Its trifluoroacetamido derivative 11g was more active than the parent compound against *S. faecium* probably due to the greater stability of the acetamido function.[29]



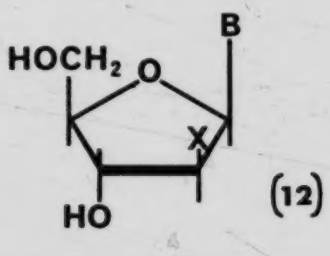
Scheme 2.1: Metabolism of 2'-halogeno-2'-deoxycytidines.

The 2'-amino **11e** and 2'-azido **11f** 2'-deoxyuridines as well as the corresponding 5'-diphosphates have also been found to be substrates for polynucleotide phosphorylase from *Micrococcus luteus*.[8]

Table 3.2 is a list of some naturally occurring and synthetic 2'-substituted arabinonucleosides. Compound **12a** is 1-β-D-arabinofuranosyluracil or spongouridine, also known as Ara-U, was first isolated from a Caribbean sponge.[11]

Watanabe *et al*[12] prepared a series of 5-substituted 2'-hydroxy- and 2'-fluoroarabinosides **12b** and **12c** as analogues of Ara-U. In contrast to Ara-U, these 5-substituted arabinosides were biologically active. Compounds of group **12b** were observed to exhibit antiviral activity in cell culture. Group **12c** suppressed herpes simplex virus type 1

Table 3.2: 2'-Substituted arabinofuranosides.



(12)

	X	B	Reference
a.	-OH	U	111
b.	-OH	5-ClU; 5-BrU; 5-IU	112
c.	-F	5-FU; 5-BrU; 5-IU	112
d.	-OH	5-FU	113
e.	-OH	C	114
f.	-F	C	115
g.	-Cl	C	116
h.	-F	5-FC; 5-ClC; 5-BrC; 5-IC	111
i.	$-NH_2$; $-N_3$	C	117

(HSV-1). A related compound 12d was also reported to possess anti-tumor activity.[113]

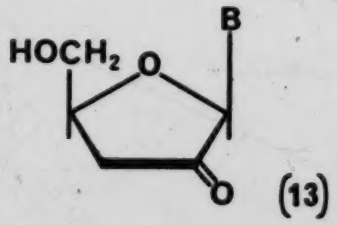
Ara-C (12e) is a potent cancer chemotherapeutic agent. The 5-fluoro analogue of Ara-C (12f) has been observed to inhibit mouse leukemia cells *in vitro*. The biological activity of the corresponding chloro analogue 11g has not

been reported.

The arabino 2'-fluoro derivatives of 5-halogenocytidines (group 12h) have been synthesized and were found to posess greater antiviral activity than the corresponding 2'-deoxy analogues. This enhanced activity was credited to the 2'-fluoro function.[11]

Arabinocytidines possessing 2'-amino and 2'-azido substituents, (group 12i), have been synthesized in an attempt to improve the stability of Ara-C toward deamination *in vivo*.[11] Both compounds were reported to be resistant to deaminase activity, effective against L1210 cells *in vivo* and were cytotoxic to some human and murine cell lines *in vivo*.

A keto derivative of uridine, 2'-keto-3'-deoxyuridine 13 has been reported[11] for which no biological data is available.



(13)

2.2.3 Analogues of 3'-Substituted Furanosides

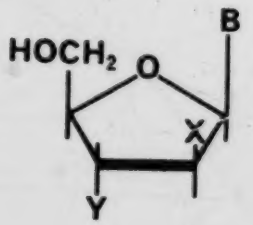
Some of the 3'-substituted nucleosides reported in the literature are shown in Tables 4.1 and 4.2. A relatively small number of 3'-substituted nucleosides have been prepared relative to other substituted nucleosides. This is probably due to the fact that the C-3' hydroxyl is required for polynucleotide formation.[119,133] Most of the compounds listed in Table 4.1 have not been evaluated biologically while some are inactive. The C-3' OH function could be replaced by an O-methyl or O-ethyl substituent with little loss of polymerization action.[88] A functional 3'-OH should assume an equatorial configuration for proper enzyme site alignment.[119,133] 1-(3'-deoxy-3'-fluoro-β-D-arabinofuranosyl)uracil has been reported to possess a twist conformation with O-ring *endo* and C-1' *exo*.[33]

Arabinocytosine (**14g**) and **14j** were reported to be potent inhibitors of replication of both murine scarcoma-180 and L1210 cells *in vivo*. Compound **14g** is a proven chemotherapeutic agent.[115]

A number of 3'-substituted xylofuranosides are listed in Table 4.2. Compound **15a** is either a poor substrate or non-substrate for cytidine deaminase.[115] Compound **15b** is active against P815 and L5187Y cell cultures.[89]

The syntheses of 3'-ketothymidines **16** have been reported but no biological data was presented.[134]

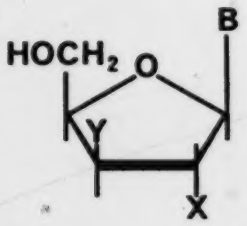
Table 4.1: 3'-Substituted arabinofuranosides.



(14)

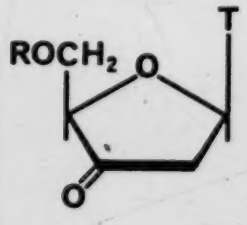
	X	Y	B	Reference
a.	-OH	-F; -Cl; -I; -OH	U	73
b.	-OH	-Br; -I; N_3; -SCN	U; C	119
c.	-OH	-I	U	120
d.	-OH	-Cl; -Br	U	121
e.	-OH	$-NH_2$; $-N_3$	U; C	122
f.	-H	-I	U	123
g.	-OH	-OH	C	115
h.	-H	-Cl; -Br	T	124
i.	-H	-Br; -I	T	125
j.	-H	-I; $-NH_2$; $-N_3$	T	126
k.	-H	$-NH_2$; $-N_3$	T	127
l	-H	-SH	T	128
m.	$-SC_2H_5$; -SCN	$-SC_2H_5$; -SCN	A	129

Table 4.2: 3'-Substituted xylofuranosides.



(15)

	X	Y	B	Reference
a.	-OH	$-SC_2H_5$	U	130, 131
b.	-OH	-F	C	115
c.	-H	-Br	C	109
d.	-H	-Cl; -Br	C	124
e.	-H	-I; $-N_3$	T	132



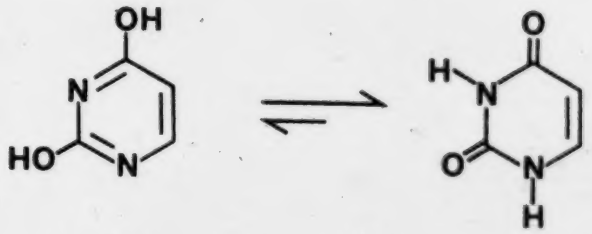
(16) a R = Tr
b R = Bz

2.3 Chemistry of Pyrimidines

2.3.1 Synthesis of 6-Substituted Uracils

The chemistry of pyrimidines has been extensively reviewed.[35,133-138] Uracil, 2,4-dihydroxypyrimidine, exists

in two tautomeric forms with the keto isomer predominating (Fig. 2.1).



(17a)

Fig. 2.1: Tautomerism of uracil.

The pyrimidine ring is a near aromatic system and resembles a benzene ring in that respect. The inclusion of the two 1,3 nitrogen atoms which are more electronegative than carbon in a benzene ring causes a distortion in the distribution of π electron density. The combined inductive effect of the two hetero atoms causes a marked electron deficiency at the 2-, 4- and 6-positions whereas the 5-position is only slightly affected. The measured π electron density at the various positions for pyrimidine are shown in Fig. 2.2.[135]

The relative electron density in a molecule determines its reactivity to electrophiles and nucleophiles. Nucleophilic substitution is favored at the 2-, 4- and 6-positions of the pyrimidine ring and is enhanced by electron withdrawing groups such as chloro and nitro substituents. Halogens are readily displaced by nucleophiles such as water, amines,

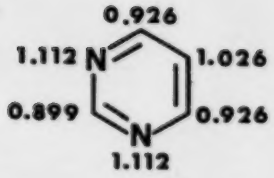


Fig. 2.2: π electron density of pyrimidine.

etc.[38]

The 5-position is only slightly electron deficient. Consequently electrophilic substitution occurs only at the 5-position of suitably activated pyrimidines. The presence of electron releasing substituents such as hydroxyl and amino functions will facilitate electrophilic substitution and deactivate the 2-, 4- and and 6-positions to nucleophilic attack.

Syntheses of 6-substituted uracils can be categorized into three main reaction types:

1. The Principle Synthetic Method

This approach involves the condensation between an amidine or urea (N-C-N fragment) and a 1,3-bifunctional three-carbon fragment possessing carbonyl or nitrile functions. An example is given in Scheme 3.1.

Malic acid 18, in the presence of fuming sulfuric acid, is converted to formylacetic acid 19 which can be condensed with urea 20 to form uracil 17.[38] Uracil derivatives substituted at C-6 with alkyl, aryl,

hydroxyl and amino groups can be prepared by careful selection of properly substituted reactants. Thus condensation of ethyl cyanoacetate 21 with urea 20 affords 6-aminouracil 22 (Scheme 3.2). Further elaboration of the 6-substituent provides other 6-substituted uracils.

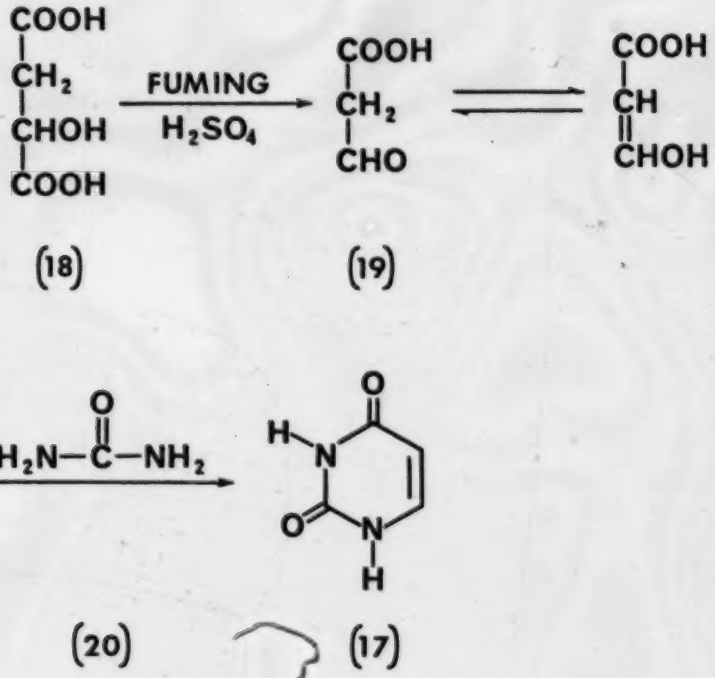
The usefulness of this reaction is sometimes limited since the condensation reaction does not proceed for some types of compounds.[38]

2. Electrophilic substitution

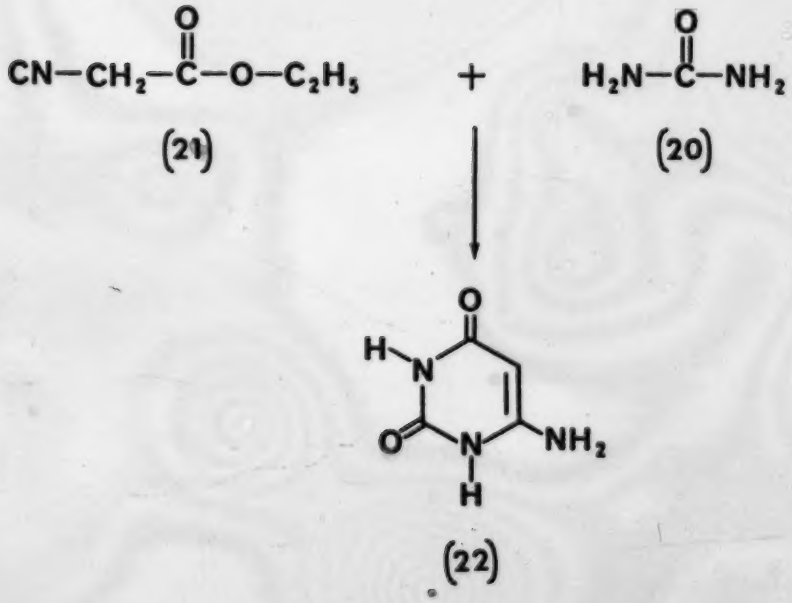
Introduction of functional groups into the C-6 position of uracil by electrophilic substitution reactions such as nitration, nitrosation, diazotization, halogenation and sulfonation are energetically unfavorable. Activation by electron releasing groups at C-5 is often a requirement. An example is afforded by nitrosation and diazotization of 5-hydroxyuracil (Scheme 3.3).

3. Nucleophilic substitution

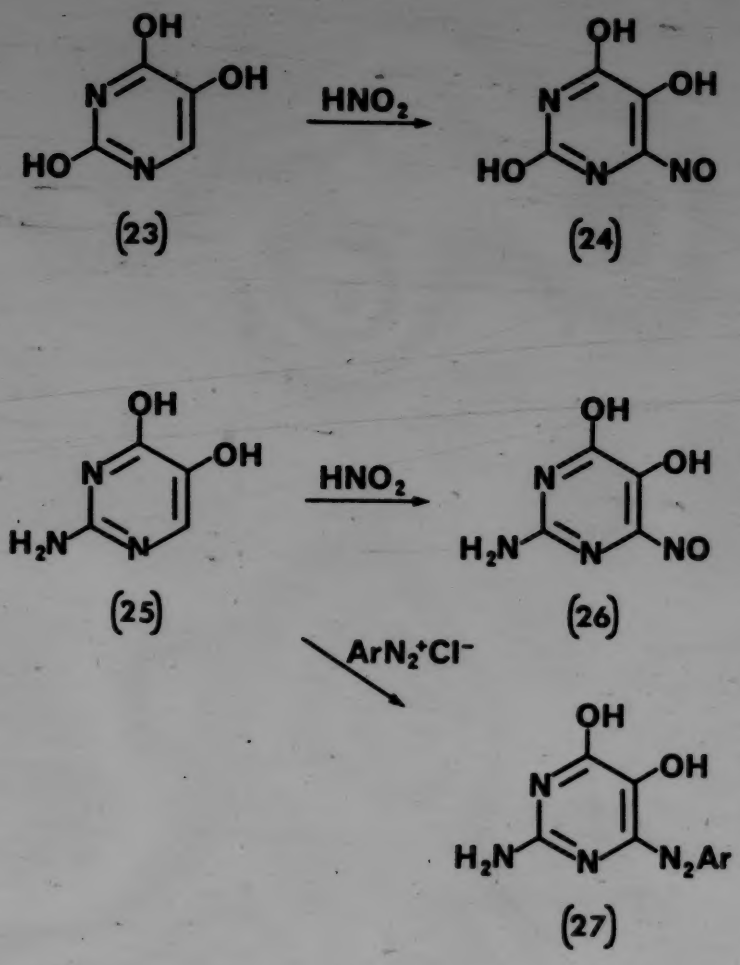
The C-6 position of uracil is electron deficient and is activated by electron withdrawing groups toward nucleophilic substitution which is considered to be the most useful synthetic method of introducing a C-6 substituent into suitably activated uracils. The mechanism of this reaction has been reviewed by Robins'[38] and is illustrated by Scheme 3.4. A stable intermediate (29a, 29b) is formed when the negative charge is delocalized on the electronegative C-4 oxygen. Expulsion of "X" occurs with the regeneration of conjugation ring



Scheme 3.1: Synthesis of uracil by the Principle Method.



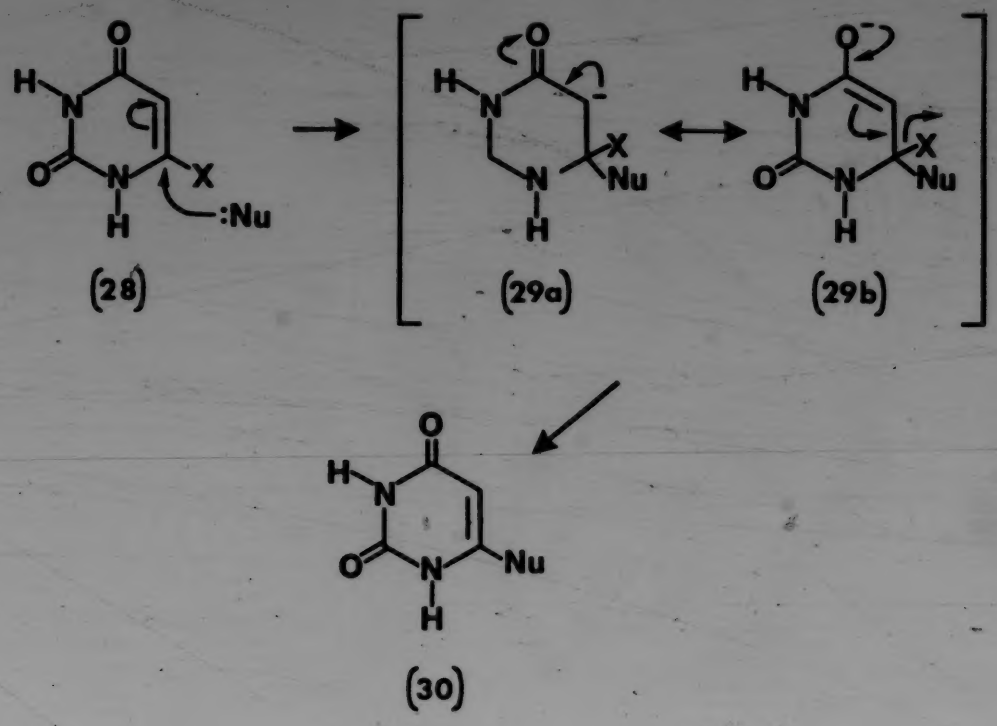
Scheme 3.2: Synthesis of 6-aminouracil.



Scheme 3.3: Electrophilic substitution in uracil.

system 30.

Uracils substituted with appropriate leaving groups at C-6 provides the radiochemist with the necessary intermediates to prepare a wide range of 6-substituted uracils. MeO, MeS, $MeSO_2$, Me_3N^+, Cl and I are some of the more commonly used leaving groups.



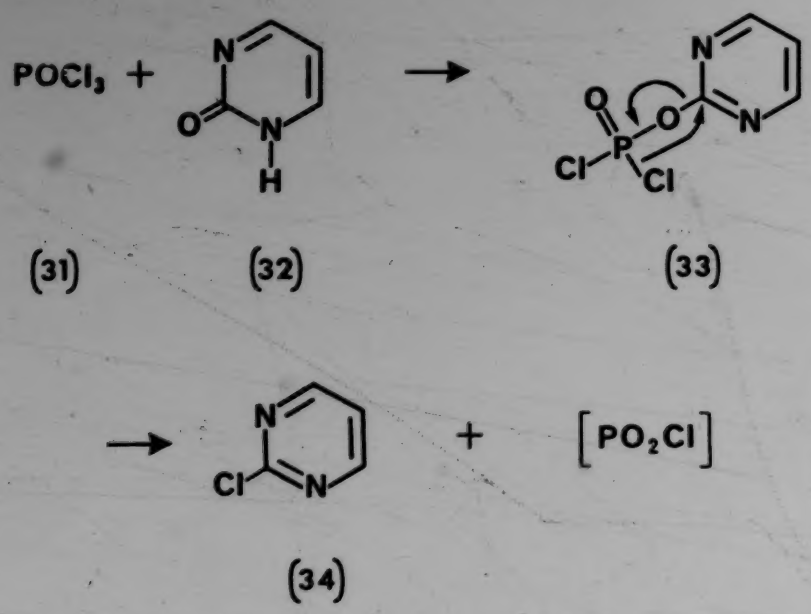
Scheme 3.4: Nucleophilic substitution in 6-substituted uracils.

Chlorine is readily introduced into the pyrimidine ring with the use of phosphorus oxychloride, 31. Thus 2-hydroxypyrimidine, 32, reacts with 31 to afford 2-chloropyrimidine, 34 (Scheme 3.5). The reaction is not a direct displacement of the C-2 oxygen by chlorine, but rather an intramolecular nucleophilic displacement involving the intermediate 33. Similarly, reaction of 2-hydroxypyrimidine with phosphorus oxybromide affords 2-bromopyrimidine and thiation of hydroxypyrimidines with phosphorus sulfides affords mercaptopyrimidines.[33]

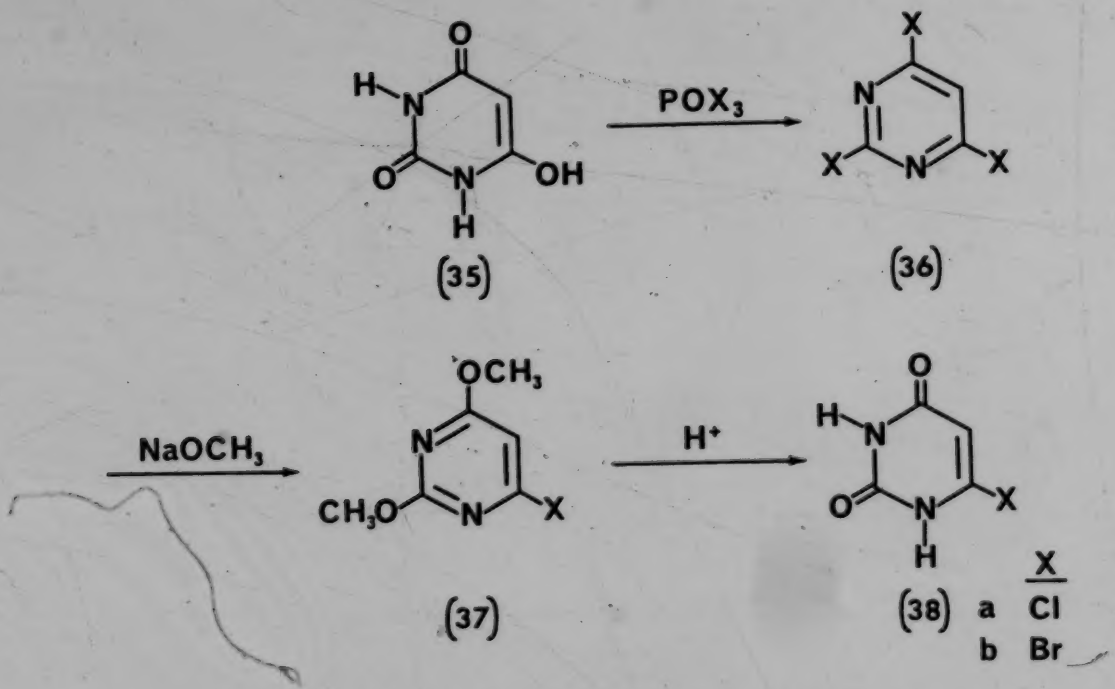
The most utilized synthetic method to prepare 6-halogenouracils is probably the halogen-for-halogen exchange reaction.[56,57,59,143] 6-Halogeno-2,4-dimethoxypyrimidines, 37, can be prepared from the reaction of sodium methoxide with 2,4,6-trihalogenopyrimidine, 36. The trihalogenopyrimidines, 36, are prepared from the reaction of a phosphorus oxyhalide, 31, with barbituric acid, 35, as described earlier. Hydrolysis of 37 using dilute mineral acid in acetic acid affords 6-halogenopyrimidines (38a, 38b)[56,57] (Scheme 3.6).

Synthesis of 6-azido-, 6-fluoro- and 6-iodouracil by acid hydrolysis of the corresponding 2,4-dimethoxy-6-substituted uracils has been unsuccessful with barbituric acid as the sole product. The ready displacement of these C-6 substituents is the result of stabilization of the positive charge after protonation of one of the ring nitrogens.[56,144] However, the lability of the C-6 fluoro atom has been utilized for the synthesis of 6-amino-, 6-substituted amino-, 6-alkoxy- and 6-thionucleosides.[56]

Ueda *et al*[57] prepared 6-cyanouridine and 6-cyanocytidine using cyanide anion as the nucleophile in DMF (Scheme 3.7). The product was reported to possess a *syn* conformation. Nucleophilic attack by cyanide anion at the C-6 position of uracil affords 5,6-dihydro-5-bromo-6-cyanouridine 40 as an intermediate. 6-Cyanouridine 41 is produced upon elimination of HBr. Further modification of the cyano function has afforded 6-thioamido-, 6-carboxyl-, 6-chloro-



Scheme 3.5: Chlorination of 2-hydroxypyrimidine with phosphorus oxychloride.



Scheme 3.6: Synthesis of 6-halogenouracil.

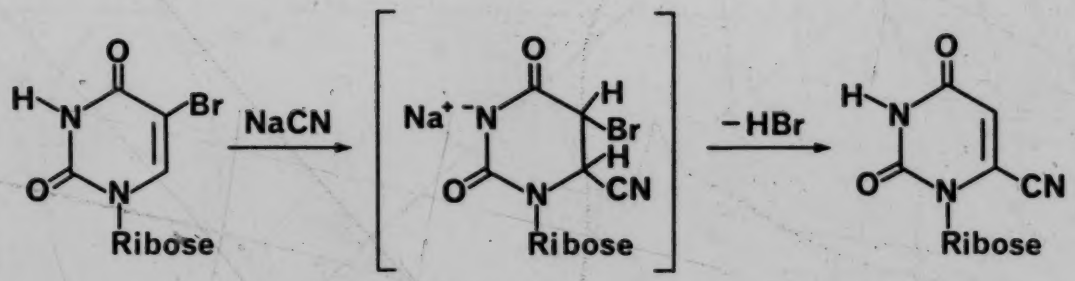
methyl-, 6-cyanomethyl-, 6-carboxymethyl- and 6-methyl derivatives of uridine. 6-Cyanocytidine and cytidine-6-carboxylic acid have been prepared using similar reactions.

Many 6-alkyl and 6-aryl pyrimidine nucleosides have been synthesized using a sequential lithiation and alkylation reaction[139-142] (Scheme 3.8).

Lithiation of a suitably protected nucleoside using lithium diisopropylamide (LDA) occurs at the N-3 and C-6 positions of **43**.[140,141] Subsequent alkylation has been reported to occur at the C-6 position of **44**. Aliphatic and aromatic aldehydes react with the lithiated derivatives to afford the corresponding C-6-carbinols. Reaction with ketones yields substituted alcohols. Acid chlorides with no α-hydrogen react effectively to give the corresponding C-6-ketones. Ethyl formate reacts to give an unstable C-6-aldehyde which can be converted to the 6-hydroxymethyl derivative upon reduction. Attempted acylation with other esters such as ethyl benzoate and ethyl acetate afforded only uridine. Reaction with alkyl halides affords the corresponding 6-alkyl derivative.

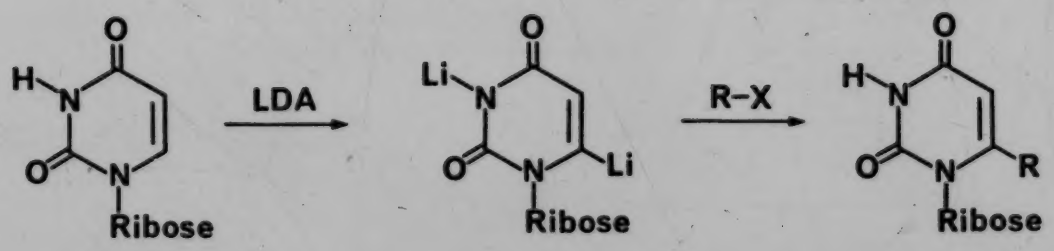
Other lithiating agents such as 2-lithio-1,3-dithiane[139] and *n*-butyllithium[142] have been used with success.

A methyl group can also be regioselectively introduced into the C-6 position of uridine with protected sugar by sequential irradiation, mesylation and treatment with NaH (Scheme 3.9).[143]



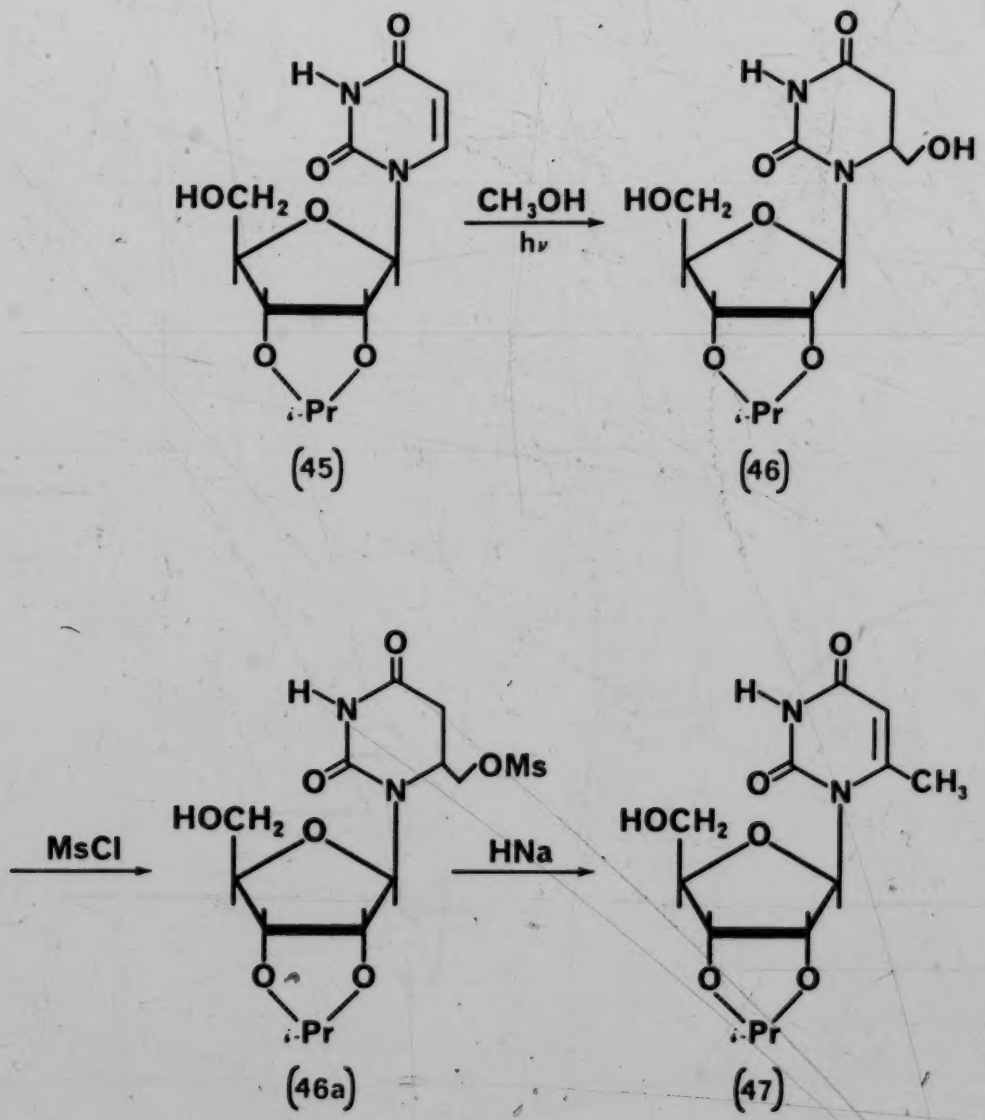
(39) (40) (41)

Scheme 3.7: Synthesis of 6-cyanouridine.



(42) (43) (44)

Scheme 3.8: Synthesis of 6-alkyluracil using lithium diisopropylamide and alkyl halides.



(45) (46) (46a) (47)

Scheme 3.9: Synthesis of 6-alkyluridine by irradiation.

2.3.2 Synthesis of 2'-Deoxy-2'-Substituted Uridines

Most chemical modifications of the C-2' position of nucleosides involve reactions employing the intermediate 2,2'-anhydronucleoside. Other synthetic methods have been reported but are usually multi-step reactions requiring blocking and deblocking of reactive groups. The chemical yields are usually low. Many advantages describing the use of 2,2'-anhydronucleosides have been cited.[46] The elimination of protective procedures, control of stereochemical configuration and introduction of the label in the final step of a reaction sequence are important in designing radiochemical syntheses.

Evidence for the 2,2'-anhydro bond was first reported by Brown *et al.*[47] The chemistry was later developed by Fox and co-workers. 2,2'-Anhydronucleosides can be prepared by a number of methods:[33,48]

1. Reaction of base with appropriately derivatized nucleosides to afford the corresponding 2,2'-anhydronucleosides (Scheme 4.1)

   The C-2'-OH is usually functionalized with a good leaving group such as *p*-toluenesulfonyl (Ts)[47,48] or methylsulfonyl (Ms).[33,52] In some cases the C-2'-OH does not need to be derivatized.[46] The C-3'-OH and C-5'-OH are usually protected by acetyl (Ac)[46] or Ms[51] functions. The triphenylmethyl (Tr) group has been used only to protect the C-5'-OH.[52]

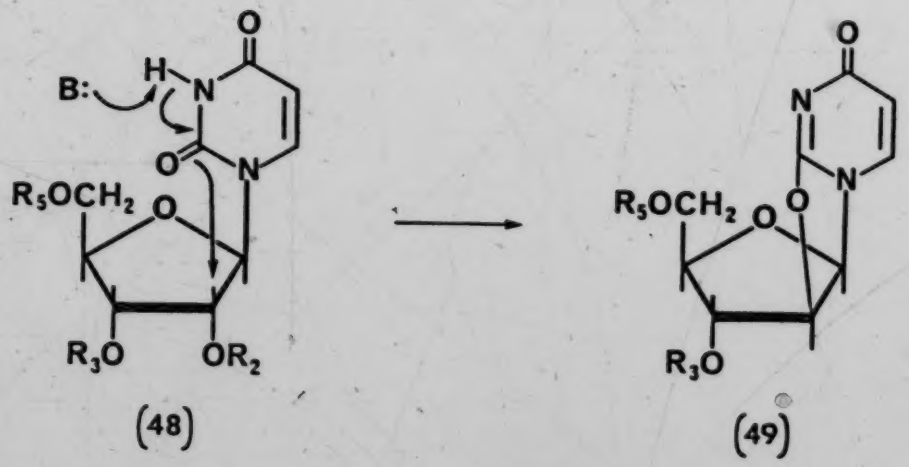
The 2,2'-anhydronucleoside analogues of cytidine,[149,152] 6-azauridine[153] and sulfur-bridged 2,2'-anhydrothymidine[154] have been similarly prepared.

2. Reaction of uridine with diphenylcarbonate and sodium bicarbonate

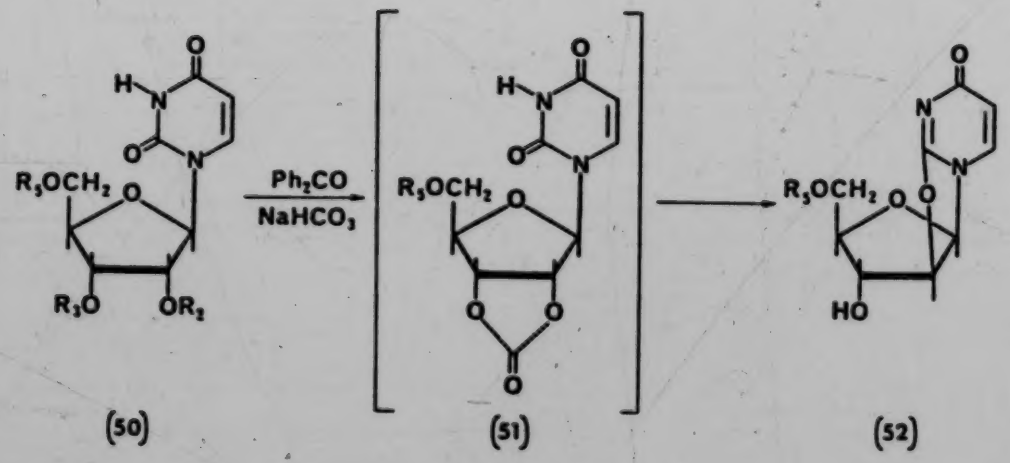
Reaction of diphenylcarbonate and sodium bicarbonate with uridine affords 2,2'-anhydrouridine[155] (Scheme 4.2). Sodium bicarbonate catalyzes the ester exchange between diphenyl carbonate and the furanose ring. This reaction is of special value because protection or the presence of a good leaving group is not required.

3. Miscellaneous reactions

2,2'-Anhydro derivatives of 5'-O-trityluridine and its 6-aza analogue can be prepared by treatment with carbonyldiimidazole in DMF followed by heating with imidazole[157] or by the use of thiocarbonyldiimidazole.[158] Treatment of uridine with phosphorus oxychloride in ethyl acetate in the presence of one equivalent of water under reflux affords 3',5'-di-O-acetyl-2,'2'-anhydrouridine.[159] Reaction of cytidine with Vilsmeier-Haack reagent, prepared from phosphorus oxychloride and DMF, affords 2,2'-anhydrocytidine.[160] 2,2'-Anhydrocytidine can also be produced by treating cytidine with thionyl chloride.[161] Sulfuryl chloride reacts with uridine and cytidine to yield both 2,2'-anhydro and 5-chloro derivatives of uridine and cytidine.[163]

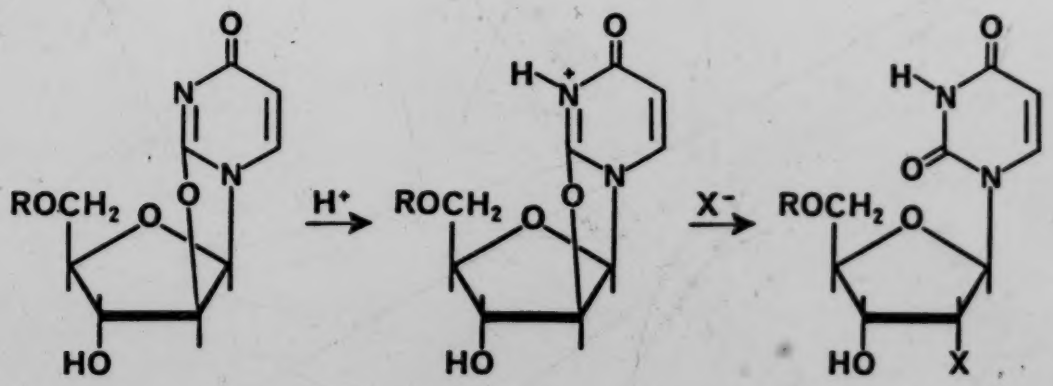


Scheme 4.1: Synthesis of 2,2'-anhydrouridines.



Scheme 4.2: Synthesis of 2,2'-anhydrouridine from uridine using diphenylcarbonate and sodium bicarbonate.

The opening of the 2,2'-anhydro bond provides the most direct synthetic approach to C-2' substituted nucleosides. 2'-Fluoro **55a**, 2'-chloro **55b**, 2'-bromo **55c** and 2'-iodo **55d** derivatives of uridine and thymidine can be synthesized from the corresponding 2,2'-anhydronucleosides and the appropriate halide in good yield with retention of C-2' *ribo* configuration.[73,73,163,164] Protonation at N^3 position of **54** (Scheme 4.3) facilitates nucleophilic attack by halide ion at the C-2' position.



(53) (54) (55) a b c d

Scheme 4.3: Syntheses of 2'-halogenonucleosides from 2,2'-anhydronucleosides.

The reaction does not proceed when sodium iodide is used alone.[73] Reaction of 2,2'-anhydrouridine with methyl iodide in DMF at room temperature was reported to afford 2'-deoxy-2'-iodo-N^3-methyluridine indicating an electronic

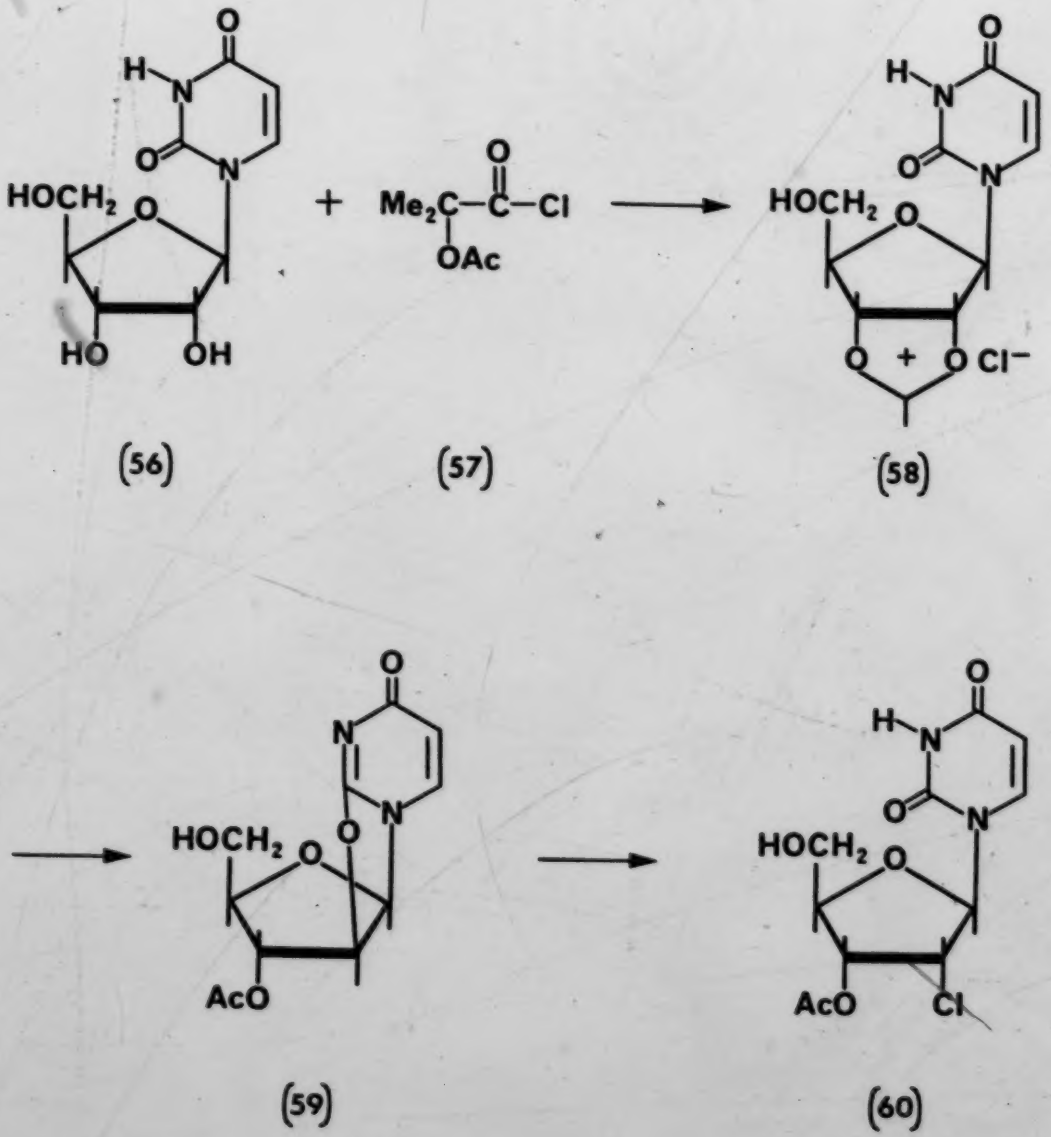
equivalence of protonation by methylation.'[53] In a comparable reaction the 2'-chloro derivative **55b** has been synthesized by heating the corresponding hydrochloride salt of the 2,2'-anhydronucleoside in dioxane.'[53] Similarly, 2'-fluoro-2'-deoxyuridine **55a** has been prepared by heating the hydrofluoride salt of 2,2'-anhydrouridine in DMF.'[4]

Along similar lines, the synthesis of 2'-deoxy-2'-fluorocytidine has been effected by treatment of the corresponding 2,2'-anhydro derivative with potassium fluoride with a crown ether in carefully dried DMF.'[7]

The intermediate 2,2'-anhydronucleoside can also be generated *in situ* during a reaction. A classical example is the syntheses of 2'-deoxy-2'-iodouridine and thymidine by Brown *et al.*'[3]

The chlorinating agent, 2-acetoxyisobutyryl chloride **57** has been used neat or in various solvents at 80°C for the synthesis of 2'-chloronucleosides.'[4] Treatment of uridine with **57** led to 3'-O-acetyl-2'-chloro-2'-deoxyuridine **60** *via* the 2',3'-O-acetoxonium ion **58** and the 2,2'-anhydronucleoside **59** (Scheme 4.4). Acetonitrile, acetic acid and nitromethane are the preferred solvents. When nitromethane is used the crystalline hydrochloride salt of 2,2'-anhydronucleoside **59** could be isolated. A similar reaction of **57** with cytidine yielded 2,2'-anhydrocytidine in good yield.'[44]

Reaction of purines with **57** initially affords the acetoxonium ion which then undergoes opening by halide to give the isomeric *trans* haloacetates.'[47]



(56) (57) (58)

(59) (60)

Scheme 4.4: Synthesis of 3'-O-acetyl-2'-chloro-2'-deoxy-uridine.

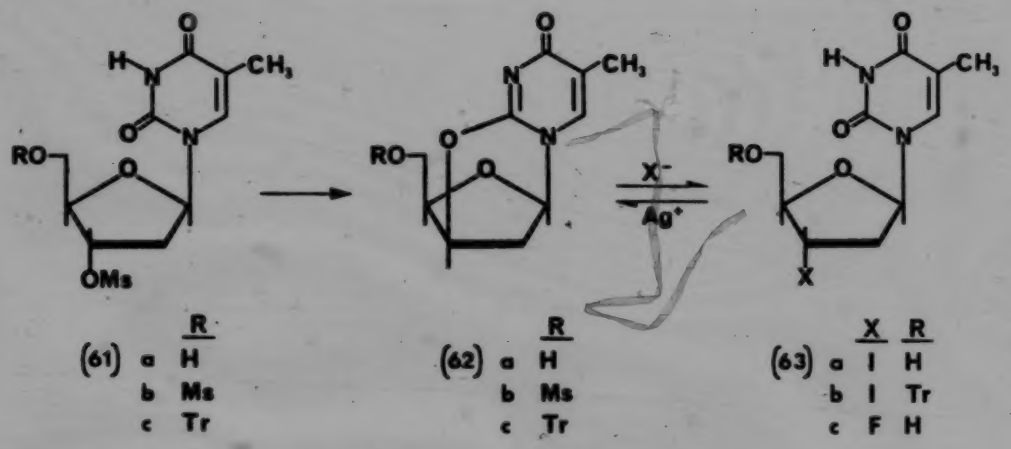
Preparation of 2'-amino-2'-deoxynucleosides is usually accomplished by reduction of the 2'-azido derivatives which can be readily synthesized by the nucleophilic reaction of azide anion with either pre-formed[85] or *in situ* generated[86] 2,2'-anhydronucleosides.

2'-Deoxy-2'-thiouridine can be synthesized by sequential reactions of 2,2'-anhydrouridine with thioacetic acid in DMF or dioxane and mild hydrolysis.[92]

2.3.3 Synthesis of 3'-Substituted Pyrimidine Nucleosides

Most synthetic approaches to 3'-substituted nucleosides involve 2,3'-anhydronucleosides the chemistry of which has been reviewed by Fox'[48] and Moffat.'[37] The first successful synthesis of 3'-deoxy-3'-iodothymidine from 3'-O-mesylthymidine with retention of C-3' configuration was reported to proceed *via* the intermediacy of a 2,3'-anhydronucleoside.'[37] The general reaction scheme is represented in Scheme 5.1.

The C-5' OH is generally protected and the C-3' OH is derivatized with a good leaving group as discussed previously for the preparation of 2,2'-anhydronucleosides. The use of an iodo substituent as a leaving group has also been documented.'[48] The formation of the 2,3'-anhydro bond is base catalyzed. Various inorganic bases such as ethanolic ammonia,'[35] sodium hydroxide or refluxing water'[49] or organic bases such as 1,5-diazobicyclic-[5.4.0]-undec-5-ene (DBU),'[70] among others, have been reported. Specialized reagents such as Rydon reagent (methyltriphenoxyphosphorium



Scheme 5.1: Synthesis of 3'-deoxy-3'-iodothymidine.

iodide, PhO_3P^+–MeI^-)[111] and triphenylphosphine/diethylazodicarboxylate/methyl iodide,[133] among others, have been used for the syntheses of 2,3'-anhydronucleosides.

Unprotected nucleosides such as thymidine, 2'-deoxyuridine and 5'-fluoro-2'-deoxyuridine have been converted into their corresponding 2,3'-anhydronucleosides using diethyl-(2-chloro-1,1,2-trifluoroethyl)amine in DMF. The reaction proceeds via a reactive oxyimmonium species (63, X = Et_2N^+=C(OR)CHFCl) which is readily displaced upon attack by the O^2 atom of the pyrimidine ring.[133]

2.3.3.1 Syntheses of 3'-Halogenonucleosides

The syntheses of 3'-halogenonucleosides have been accomplished by the following methods:

1. Reaction of 2,3'-anhydronucleosides with nucleophiles

The opening of the 2,3'-anhyro bond by a nucleophile provides a covenient means of preparing 3'-halogenonucleosides. Thus, 3'-fluoro- and 3'-chlorothymidine[72] and 3'-deoxy-3'-iodo-N³-methylthymidine and uridine[68] have been prepared in this way.

Direct displacement of a 3'-O-mesyl substituent in thymidine by iodide affords 3'-iodothymidine *via* the 2,3'-anhydronucleoside.[28] Reaction of 5'-O-tritylthymidine with Rydon reagent in DMF at room temperature affords 3'-deoxy-3-iodothymidine presumably by way of a 2,3'-anhydronucleoside intermediate.[71] On the other hand, 3'-deoxy-3'-iodoxylofuranoside is the main product obtained upon treatment of 2',5'-di-O-trityluridine with Rydon reagent indicating a direct displacement of the 3'-trityl function rather than initial formation of the 2,3'-anhydro bond.[71,73]

There are limitations in using 2,3'-anhydronucleosides for the synthesis of 3'-substituted nucleosides. Protection of C-5' OH is usually required since formation of a 2,5'-anhydro bond is more facile than formation of a 2,3'-anhydro bond. For example 3'-O-mesyluridine and 3'-O-tosyluridine afford 2,5'-anhydrouridine under mildly basic conditions. On the other hand when sodium *t*-butoxide is used 2,3'-anhydrouridine is formed[74] which then isomerizes to the corresponding 2,2'-anhydronucleoside under more vigorous conditions, presumably

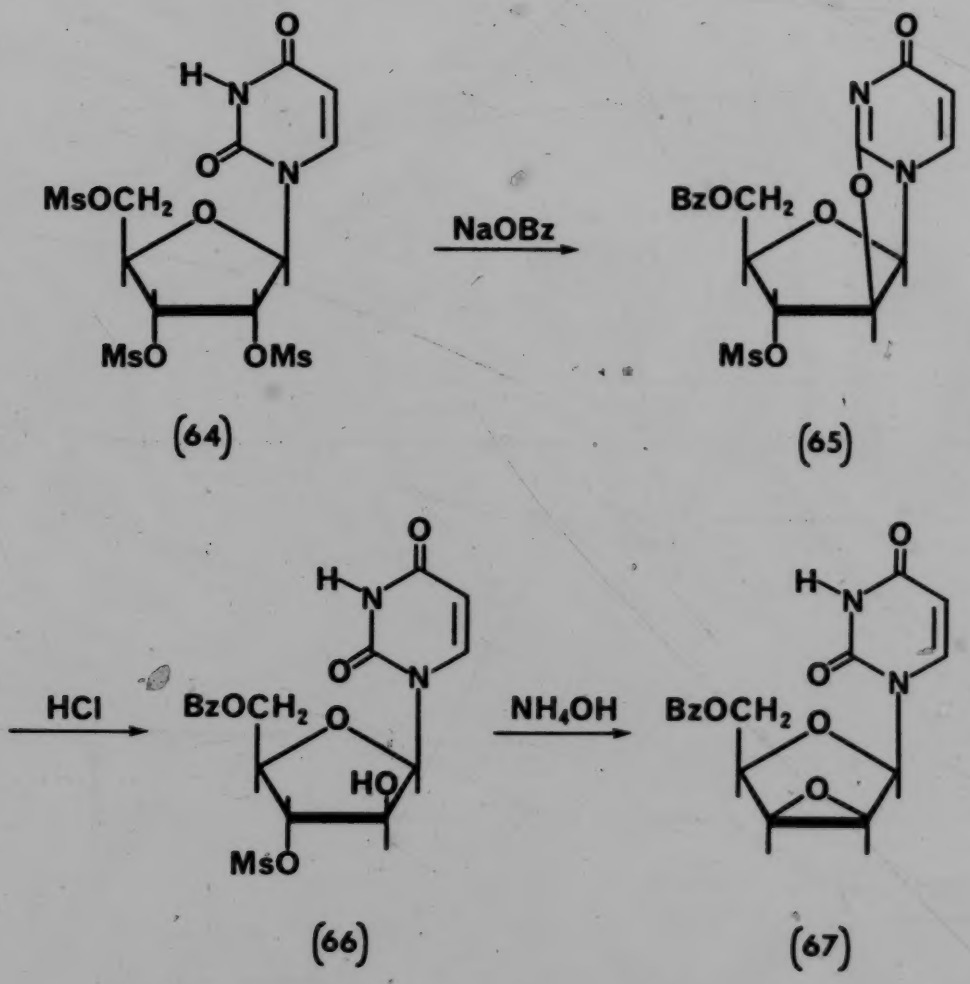
via the 2',3'-epoxide.[175,176] In contrast, the anhydronucleosides of cytidine have been found to be stable under alkaline conditions.[177] Another example of isomerization is the unprotected 2,3'-anhydrouridine which reacts with methyl iodide to give exclusively the 5'-iodonucleoside probably *via* rearrangement of the 2,3'-anhydronucleoside to its 2,5'-anhydro isomer.[165]

2. Nucleophilic opening of 2',3'-lyxoepoxides

Another approach to 3'-halogenoarabinonucleosides is the reaction of nucleophiles with 2',3'-lyxoepoxides. These epoxides are readily prepared from suitably derivatized 3'-O-mesylthymidine,[69] xylofuranosides,[178-181] ribofuranosides,[182-184] or arabinofuranosides.[184] The preparation of a 2',3'-lyxoepoxide from 2',3',5'-tri-O-mesyluridine **64** is illustrated in Scheme 5.2. The reaction proceeds *via* the 2,2'-anhydronucleoside **65** and arabinofuranosyl **66** intermediates.

Treatment of *lyxo* epoxides **68** with halide anions in the presence of HCl usually leads almost exclusively to the 3'-deoxy-3'-halogenoarabinofuranosyl derivatives **69** with Walden inversion of configuration at C-3' (Scheme 5.3).

In the uridine series, 3'-fluoro-, 3'-chloro-, 3'-iodo-[73,128] and 3'-bromoarabinonucleosides[129,131] have been synthesized using this synthetic approach. The reaction has been extended to the cytosine[69] and adenine[84] series. The 2',3'-lyxoepoxide can also be

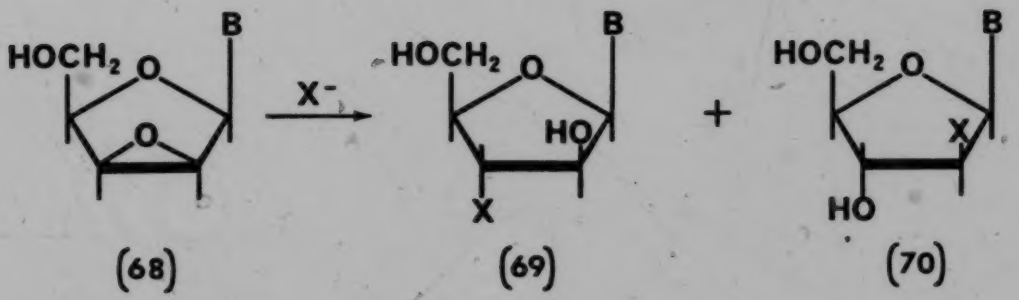


(64) (65) (66) (67)

Scheme 5.2: Synthesis of 2',3'-lyxoepoxide.

generated *in situ* as an intermediate during the reaction.[87]

Reports of the formation of the *xylo* isomer 70 are inconsistent. It was undetected by some researchers,[11,19,20,22,88] while others reported yields of minor quantity,[84,85] 4.8%[20] and 18%.[84]

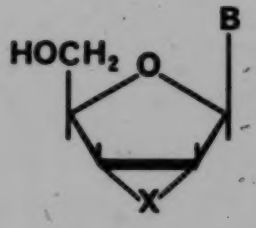


(68) (69) (70)

	B
a	U
b	C
d	T

Scheme 5.3: Synthesis of 3'-deoxy-3'-halogenoarabino-furanosides.

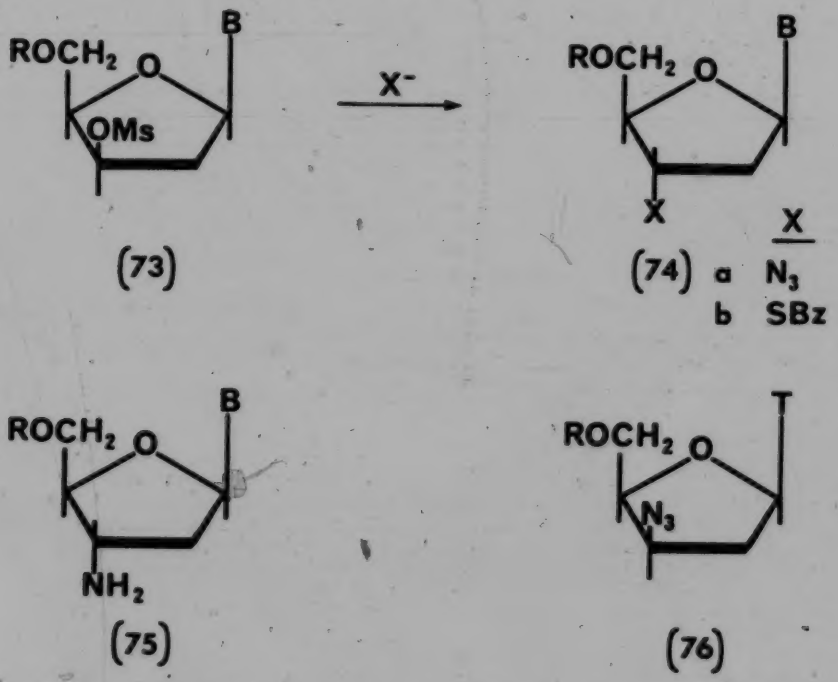
Nucleophilic opening of the *ribo* epoxides, 71 is restricted to the purine series.[1,4,6,7,9] The *ribo* epoxides of pyrimidines rearrange readily to the corresponding 2,2'-anhdyro derivatives.[1,7,10,12] One exception is the uridine ribothioepoxide 72 which has been reported to be stable.[1,3]



	X
(71)	O
(72)	S

2.3.3.2 Synthesis of 3'-Nitrogen and 3'-Sulfur Substituted Nucleosides

3'-Azidothymidine 74a can be prepared by treatment of preformed 2,3'-anhydrothymidine[114] or its 5'-O-trityl derivatives[115] with azide salts in DMF. Direct displacement of the *threo* mesylate of thymidine 73 with azide anion[116,117] also affords 74a (Scheme 5.4). Catalytic reduction of 74a then gives the corresponding 3'-amino derivative, 75.



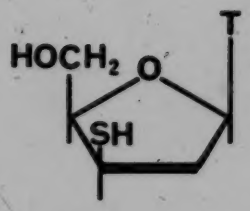
Scheme 5.4: Synthesis of 3'-azido and 3'-amino-3'-deoxy-nucleosides.

The configurational isomer with the "up" 3'-azido function 76 has been prepared by the reaction of triphenylphosphine and diethylazodicarboxylate with 5'-O-trityluridine or 2',5'-di-O-trityluridine in the presence of hydra-

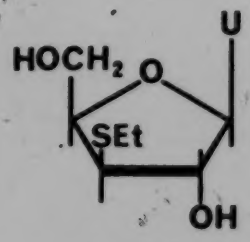
zoic acid.[132]

Direct opening of epoxides 68a[122] and 68b[119] by azide anion affords the corresponding 3'-azido derivativs (69a, 69b). The 2',3'-epoxides of uridine[134] and 6-azauridine[133] have been opened directly with ammonia to generate the 3'-amino derivatives 74b.

Relatively few 3'-sulfhydryl nucleosides have been prepared since there is a tendency for these compounds to convert to the corresponding disulfides.[137] The 3'-thio *xylo* nucleoside of thymidine (77) has been synthesized by careful hydrolysis of the corresponding 2,3'-thioanhydronucleoside.[138] The corresponding 3'-ethylthioxylo derivative of uridine 78 has been prepared by the reaction of mercaptide anions with 2,3'-anhydrouridine. The *xylo* configuration suggests the formation of an intermediate *ribo* epoxide.[11]

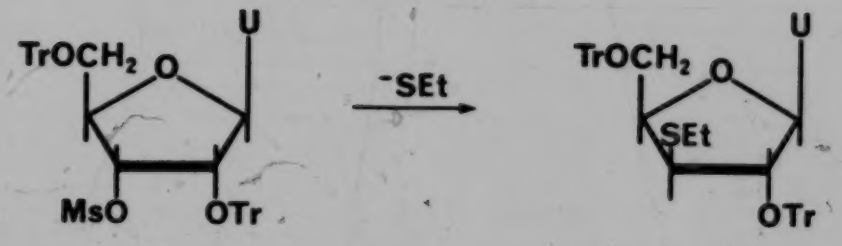


(77)



(78)

Direct nucleophilic displacement of sulfonate esters by sulfide has been used successfully to prepare the 3'-ethylthio derivative of uridine 80 in moderate yield from 3'-O-mesyl-2',5'-di-O-trityluridine 79[11,119,134] (Scheme 5.5). The same compound has also been prepared in high yield from



(79) (80)

Scheme 5.5: Synthesis of 1-(2',5'-O-trityl-3'-deoxy-3'-ethylthio-β-arabinofuranosyl)uracil.

3'-,5'-di-O-acetyl-2,2'-anhydrouridine, presumably *via* a nucleophilic attack by the mercaptide anion at C-3' of the 2',3'-O-acetoxonium ion.[138]

Application of nucleophilic epoxide opening to synthesis of 3'-thiocyanonucleoside has also been successful. Thus the opening of uridine[133] and cytidine[137] 2',3'-lyxoepoxides with thiocyanate ion affords the corresponding 3'-thiocyano derivatives as the major products.

2.4 Radioisotopes of Chlorine and Bromine

Halogens have been described as "organic isotopes"[139] which are capable of replacing a hydrogen atom or a hydroxyl function (OH) in most organic molecules. In this respect, halogen-labelled biochemicals have been widely used in

biochemistry and nuclear medicine as investigative tools. Some of their physicochemical properties are compared with those of hydrogen, methyl (CH_3), OH, and carboxyl (COOH) in Table 5.1.

Table 5.1: Physicochemical properties of some common substituents.

Substituent	Bond Strength*	Electron Affinity†	STERIMOL Parameters (Å)‡					
			L	B_1	B_2	B_3	B_4	op¶
-H	81	18.4	2.06	1.00	1.00	1.00	1.00	-
$-CH_3$	45	24.8	3.00	1.52	1.90	1.90	2.04	1-4
-OH	-	42.1	2.74	1.35	1.35	1.35	1.94	1-4
-COOH	-	-	3.91	1.60	1.60	2.36	2.66	1-2
-F	128	79.6	2.65	1.35	1.35	1.35	1.35	-
-Cl	95	83.2	3.52	1.80	1.80	1.80	1.80	-
-Br	67	77.4	3.83	1.95	1.95	1.95	1.95	-
-I	50	70.8	4.23	2.15	2.15	2.15	2.15	-

*C-X bond strength in Kcal M^{-1}''
†Electron affinity in Kcal gm^{-1} ions''
‡Reference 199
¶Opposite pair of B parameters, i.e. 1-4 means that B_1 and B_4 are in opposite direction

In Table 5.1, the carbon-halogen bond strengths are arranged in a descending order of bond energy with C-F > C-Cl > C-Br > C-I. Electronegativity follows the same order as bond strength, with fluorine being the most electronegative and iodine the least so among the lighter

halogens.

The sizes of the substituents are in the reverse order of bond strengths as indicated by the STERIMOL steric parameters. *L* represents the overall length of the substituent (the farthest extension in the X-direction of the molecule i.e. the X-coordinate of the tangential plane to the van der Waals radii perpendicular to the X-axis) and is made up of the partial bond lengths of the substituent and a benzene carbon plus the van der Waals radius of the substituent. B_1, B_2, B_3 and B_4 can be visualized as the minimal size of a close fitting box around the substituent. Hydrogen and halogen substituents are considered to be spherical in shape and their *B* parameters and van der Waals radii are numerically equivalent.

An iodine and bromine substituent resemble a methyl group in size but are considerably more electronegative. Chlorine forms a stronger bond with carbon and is more electronegative but less bulky than bromine and iodine. Flourine is the most electronegative of the halogens and is also the smallest in size.

With advancement in isotope production technology many radioisotopes with potential medical application are available. The most promising ones are neutron-deficient positron (β^+) emitters with short physical half-lives (T1/2), produced in accelerators and cyclotrons. A short half-life has been defined as four days in order to include ^{111}In and ^{67}Ga, but to exclude ^{131}I which is less than ideal for

in vivo applications.[200] Several advantages are often associated with the use of short-lived positron emitters:

3. High specific activities and smaller doses
4. Frequent dosing is possible
5. Low radiation dose to patient
6. Less contamination problem
7. Dynamic information from a steady state
8. Quantitation is more easily performed.

The fact that they are short-lived, radioactive and usually only small quantities are required at a time dictates specialized handling techniques:

1. Short synthetic reaction times
2. Micro scale reactions
3. Specially developed synthetic procedures
4. Radiation protection of operators.

Among the radioisotopes of chlorine and bromine, ^{34m}Cl and ^{77}Br are potentially useful medical isotopes. For reason of availability ^{34}Cl and ^{82}Br were used in the present investigation as suitable models for ^{34m}Cl and ^{77}Br.

2.4.1 Isotopes of Chlorine

The element chlorine (atomic number 17) consists of two naturally occurring isotopes, ^{35}Cl (75.77%) and ^{37}Cl (24.23%) and eleven radioisotopes.[201] A summary of their radioisotope data is presented in Table 5.2.[201]

Two radioisotopes, ^{38m}Cl and ^{40}Cl (T1/2 = 0.10 sec), are not included in the table. ^{38m}Cl is described as being

Table 5.2: Radioisotope data of chlorine.[28]

Mass No. (A)	Half-life*	Type of decay (d) Abundance (%) Thermal n cross section, barns (b)	Major Radiations: Approx. energies (MeV) Relative intensities
32	298 ms	(d) β^+ α (≅0.01%)	β^+ 9.47 (50%) 4.75 (25%) γ 2.24 (100%) 4.77 (22.3%)
33	2.51 s	(d) β^+	β^+ 4.51 γ 0.84 (96%) 1.966 (100%) 2.866 (100%)
34	1.53 s	(d) β^+	β^+ 4.50
34*m*	32.0 m	(d) β^+ (53%) IT (47%)	β^+ 2.47, 1.35 e- 0.142 γ with β^+ 1.176 (27%) 2.128 (100%) 3.304 (26%) γ with IT 0.146 (74%)
35		(%) 75.77 (b) 43	
36	3X10^5 y	(d) β^- (98.1%) EC (1.9%)	β^- 0.7087
37		(%) 24.23 (b) 0.428 (to ^{37}Cl) 0.005 (to ^{37m}Cl)	
38	37.3 m	(d) β^-	β^- 4.913 (57.6%) γ 1.642 (73.8%) 2.168 (100%)
39	56 m	(d) β^-	β^- 1.91 (85%) γ 0.250 (86.6%) 1.267 (100%) 1.517 (71%)

Table 5.2: Radioisotope data of chlorine. (cont'd)

Mass No. (A)	Half-life*	Type of decay (d) Abundance (%) Thermal n cross section, barns (b)	Major Radiations: Approx. energies (MeV) Relative intensities
40	1.35 m	(d) β^-	β^- 7.5 γ 1.461 (100%) 2.622 (19.6%) 2.840 (38.6%)
41	34 s	(d) β^-	β^- 3.80 γ

*ms = millisec; s = sec; m = min; y = yr

element probable and mass number certain or probable. Two chlorine isotopes with mass number 40 have been reported.[20] ^{40}Cl with T1/2 = 1.35 min is listed in Table 5.2. The existence of ^{40}Cl with T1/2 = 0.01 sec is uncertain with insufficient supportive data.[20]

Most radioisotopes of chlorine are either very short-lived or hard β^- emitters (Table 5.2). ^{34m}Cl is the only potentially useful chlorine isotope for *in vivo* applications. ^{34m}Cl (T1/2 = 32.0 min) undergoes isomeric transition (IT) to ^{34}Cl (T1/2 = 1.53 sec) which then decays rapidly by β^+ emission to the excitated state of ^{34}S which returns to its ground state by photon emission.[20]

The production of ^{34m}Cl requires bombardment of suitable target materials by high energy particles generated by a cyclotron or an accelerator. Several nuclear reactions

have been employed utilizing high energy particle beams. Machulla *et al*[202] reported the use of a (p, pn) reaction on ^{35}Cl with a 15% carrier-free ^{34m}Cl yield. Other researchers published inorganic yields of 20 to 68% using alkylhalides as target materials.[203,204] Successful production reactions of $^{31}P(\alpha, n)^{34m}Cl$ and $^{24}Mg(^{12}C, pn)^{34m}Cl$ have also been documented.[201]

Chlorine-36 is available commercially. It decays by β^- to ^{36}Ar. It also decays by orbital electron capture (EC, 1.17%) to ^{36}S followed by X-ray emission.[205] The usual production method is by an (n, γ) reaction on ^{35}Cl using thermal neutrons. Since it has a long half-life (T1/2 = 3 X 10^5 yr) practical production is strictly limited to extended uranium pile reactor irradiation at high neutron flux densities. Several nuclear reactions occur upon neutron irradiation. The most important ones are $^{35}Cl(n, \gamma)^{36}Cl$ and $^{35}Cl(n, p)^{35}S$.[206] The competing reaction of ^{35}S production necessitates periodic separation of the products.

Low specific activity is an intrinsic property of ^{36}Cl. Many attempts have been made to prepare carrier-free or no-carrier-added ^{36}Cl. Bell and Stöcklin[206] applied a combined (n, γ) and Szilard-Chalmers reaction[207] of the recoiling nuclei upon activation and concentrating the liberated isotopes to obtain 15% yield (37 GBq) of carrier-free ^{36}Cl-chloride from hexachlororhenates after 35 days of irradiation at a thermal neutron flux of 6 X 10^{13} n cm^{-2} sec^{-1}.

Production reactions using high energy particle beams such as $^{35}Cl(d, p)^{36}Cl$ and $^{34}S(d, 2n)^{36}Cl$ are feasible, but the long half-life of ^{36}Cl precludes any such application.[208]

2.4.2 Isotopes of Bromine

The stable isotopes of bromine (atomic number = 35) possess mass numbers of 79 (50.69%) and 81 (49.31%).[209] Their nuclear data are listed in Table 5.3.

Twenty-nine radioisotopes of bromine are listed in *Table of Isotopes*.[209] Radioisotopes of bromine can be produced by a wide variety of methods[210] partly accounting for the large numbers of isotopes in existence.

1. By bombardment of stable isotopes of Se and As with charged particles (p, d, and α) accelerated to energies generally in the region of 10 to 20 MeV
2. By bombardment of stable isotopes of Br, Kr and Rb with neutrons
3. By bombardment of Br and Rb stable isotopes with high energy photons with energy greater than the nucleon binding energy (about 8 MeV) of the target nucleus
4. By bombardment of a Cu target with heavy projectiles such as ^{12}C and ^{14}N
5. From the decay of radioisotopes of Se-77, 83 and 84 and from Br-80*m* and 82*m*
6. From fission of heavy nuclides (U-233, 235, 238, Pu-239, 240 and Th-232) induced by particle bombardment at low

Table 5.3: Nuclear data of ^{77}Br and ^{81}Br.[201]

Isotope	Abundance (%)	Thermal neutron cross section (barns)	
79	50.69	(to ^{80}Br)	10.8
		(to ^{80m}Br)	2.4
81	49.31	(to ^{82}Br)	0.26*
			2.72 (includes indirect capture *via* ^{82m}Br)
		(to ^{82m}Br)	2.4*

*Reference 208

to moderate excitation energies. Fission-produced bromine radioisotopes have mass numbers 82 and larger

7. From spallation-fission of moderate to heavy elements (Kr, Ta, Bi, Th and U) by high energy protons, deuterons and α-particles accelerated to 100 MeV or over.

A partial list of the radioisotopes of bromine is presented in Table 5.4. Omitted from the table are Br-70 (T1/2 = 23 sec), 71 (T1/2 = <1 min), 74 (T1/2 = 4 min), 86 (T1/2 = 55.7 sec) and 92 (T1/2 = 0.37 sec). The existence of the first four isotopes is uncertain because of insufficient evidence.[201] ^{82}Br is described as element probable and mass certain or probable.[201]

Bromine-77 (T1/2 = 57.04 hr) is one of the more useful medical isotopes of the 29 radioisotopes of bromine. It decays by EC (99.26%) and β^+ emission (0.74%) to ^{77}Se. A disadvantage of ^{77}Br is the emission of high energy photons

Table 5.4: Radioisotope data of bromine.[20]

Mass No.(A)	Half-life*	Type of decay	Major radiations: Approximate energies (MeV) Relative intensities
72	1.31 m	β^+	γ 0.862 (100%), 1.317 (24.6%)
73	3.3 m	β^+ EC	β^+ 3.7 γ 0.065 (100%), 0.336 (34%) AR (574%)
74	25.3 m	β^+ EC	β^+ 4.7 γ 0.282 (27.8%), 0.635 (100%)
74*m*	41.5 m	β^+ EC	β^+ 5.2, 4.5 γ 0.635 (100%), 0.728 (38%) AR (203%)
75	95.5 m	β^+ (75.5%) EC (24.5%)	β^+ 1.74 γ 0.286 (100%) AR (167%)
76	16.1 h	β^+ (57%) EC (43%)	β^+ 3.980, 3.440 γ 0.559 (100%) AR (175%)
77	57.04 h	EC (99.26%) β^+ (0.74%)	β^+ 0.336 γ 0.239 (100%)
77*m*‡	4.28 m	IT	γ 0.106
78	6.46 m	β^+ (92%) EC (8%)	β^+ 2.52 γ 0.614 (100%) AR (1360%)
79*m*‡	4.86 s	IT	γ 0.207
80	17.68 m	β^- (91.7%) EC (5.7%) β^+ (2.0%)	β^- 1.997 (82.2%), 1.38 (7.6%) β^+ 0.850 (2.8%) γ with β^- 0.616 (100%)
80*m*	4.42 h	IT	γ 0.037 (100%)
82	35.34 h	β^-	β^- 0.444 γ 0.698 (34.3%), 0.776 (100%) 0.828 (28.7%), 1.044 (32.8%) 1.317 (32.2%)

Table 5.4: Radioisotope data of bromine. (cont'd)

Mass No.(A)	Half-life*	Type of decay	Major radiations: Approximate energies (MeV) Relative intensities
82*m*	6.05 m	IT (97.6%) β^- (2.4%)	γ with β^- 0.776 (100%) γ with IT 0.046
83	2.39 h	β^-	β^- 0.925 (98.6%) γ 0.530 (100%)
84	31.80 m	β^-	β^- 4.626 (34%), 3.81 (20%) 2.70 (11%) γ 0.882 (100%), 1.900 (35.4%)
84*m*	6.0 m	β^-	β^- 2.2 (100%) γ 0.424 (100%), 0.802 (157%) 1.463 (97%)
85	2.87 m	β^-	β^- 2.5 γ 0.802 (157%), 0.925 (100%)
86	55.7 s	β^-	β^- 7.4 (15%) γ 1.565 (100%)
87	55.6 s	β^- β^-n (2.3%)	β^- 6.1 γ with β^- 0.532 (25.0%) 1.420 (100%), 1.476 (36.5%) 1.578 (26.8%) n 0.25 (average)
88	16.7 s	β^- β^-n (4.3%)	γ with β^- 0.775 (100%) n 0.33 (average)
89	4.37 s	β^- β^-n (12.5%)	γ 0.602 n 0.43 (average)
90	1.96 s	β^- β^-n (22.6%)	
91	0.54 s	β^- β^-n (9.97%)	

*s = sec; m = min; h = hr
AR = Annihilation radiation
‡Element certain and mass number probable

at 521 KeV (21.4%).[201] This, however, can be partially corrected by proper collimation.

Production of ^{77}Br has been reviewed by several groups of investigators.[199,201,211,212] The isotope may be produced *via* a large number of nuclear reactions. Various natural and enriched targets of As, Se, Br and Kr and various bombarding particles (p, d, ^{3}He- and α-particles) from a few MeV to tens of MeV have been used. Some of the more useful of these reactions are:

1. ^{75}As(α, 2n)^{77}Br with 29 MeV α-particles
2. ^{79}Br(p, 3n)^{77}Kr with protons of 32 to 65 MeV
3. ^{79}Br(d, 4n)^{77}Kr with 50 MeV deuterons
4. Spallation of ^{99}Mo.

Krypton-77 decays to ^{77}Br. ^{77}Kr can be produced from the reaction ^{74}Se(^{3}He, 2n)^{77}Kr.[199] The reactions are particularly useful since removal of ^{77}Kr from the target constitutes a purification step and the isolated product can be used in excitation labelling.

The isotope ^{82}Br is observed to decay almost entirely (97.6%) by β^- emission to stable ^{82}Kr. ^{82}Br can be produced by a number of methods, including bombardment with protons, deuterons, photons and neutrons, decay of ^{82m}Br, fission of heavy elements and spallation-fission of moderate to heavy elements. The most important method is the (n, γ) reaction on ^{81}Br with thermal neutrons. The amount of radioactivity produced is expressed in equation (1).

$$\text{Activity} = \sigma\phi n[1-e^{**}(-\lambda t)] \quad (1)$$

where σ = Thermal cross-section of target nuclei in cm^{-2} $atom^{-1}$ n^{-1}
ϕ = Flux of neutrons in n cm^{-2} sec^{-1}
n = Total number of target nuclei
e = Base of natural logarithm (2.718...)
λ = Decay constant of radioisotope
t = Duration of irradiation

On activation with thermal neutrons two radioisotopes, ^{82}Br and ^{82m}Br, are produced from ^{81}Br. ^{82m}Br reaches saturation activity quickly with its short half-life of 6.05 min and decays to ^{82}Br. The saturation activity of ^{82}Br induced in 1 g of natural abundance bromine has been reported to be approximately 12.95 GBq.[210] The Szilard-Chalmers reaction has also been utilized for the preparation of carrier-free ^{82}Br by several groups of researchers using organic bromides or inorganic bromates as target materials.[213-215]

2.5 Animal and Tumor Models

The general biological features of human cancers are well represented in naturally occurring cancers of small laboratory animals.[216] Most phases of tumor growth from induction through primary tumor development to metastases are closely similar in different mammalian species. The most important feature is perhaps a similarity in mechanisms of control of tumor growth which is often effected by similar agents. The major deviation from this analogy between human

cancer and that of small animals arise from species differences in body size and life span and an approximately twenty-five times difference in mean tumor volume doubling time, in accordance to the difference in species life span.[214]

Small animals such as mice offer several advantages to medical researchers. Their small physical size allows convenience and ease in handling. A large number of experimental animals can also be employed to provide statistical accuracy. Because of their short life span many time and age related physiological states can be studied over a relatively short time duration. Laboratory mice possess highly inbred isogenic qualities which permits observance of stringent control conditions so important in biomedical research and reproduction of experiments in different laboratories. Finally there is also an economic factor which small animals provide and is an important advantage in itself.

Many tumor models are available in small laboratory mice. The Lewis Lung tumor model is among those adopted by the Division of Cancer Treatment of the US National Cancer Institute for screening of chemotherapeutic agents.[214] This model of tumor exhibits faithfully characteristics of a generalized tumor disease with regular metastases[217,218] and the advantage of solid tumor. It has been widely accepted as a ideal model for general disease control by surgery, chemotherapy, adjuvant chemotherapy[219] and tumor imaging studies.[82]

The Lewis Lung carcinoma originated spontaneously as an epidermoid carcinoma (anaplastic carcinoma) in the lung of a C57BL/6 mouse first discovered by Dr. M.R. Lewis in 1951.[220] The tumor is usually maintained in BDF_1 mice (C57BL/6 female X DBA/2 male). A cell mash is prepared by serial passage of a large unulcerated portion of tumor from a healthy mouse through small gauge hypodermic needles. The reconstituted cell suspension is usually injected intramuscularly or subcutaneously.[217,219-222] As an alternate procedure, donor tumor tissue is carved into small portions measuring approximately 1 to 2 mm^3 which are injected subcutaneously.[220,223]

Transplants usually take in 100% of the cases with no spontaneous regression.[216,217,219] However, in one study 4% regression was reported[220] but this was attributed to heterogeneity among the recipients.[216] The transplants grow rapidly and do not elicit any host-immune reactions.[216,219] Different characteristic tumor growth stages such as primary tumor, number and extent of metastases are well demonstrated.[219] The tumor is relatively insensitive to chemotherapy[217] and metastasizes to lungs[217-219,223] and kidneys.[218]

The growth kinetics of Lewis Lung carcinoma have been studied in detail. The tumor grows in a Gompertzian manner[223,224] and metastasizes to the lungs of BDF_1 mice before the primary tumor reaches 0.4 g in size.[223] At 0.35 g it has an average doubling time of 2.4 days, and at 6.4 g it doubles its size in about 23 days.[223] If the inoculation

point is intramuscularly into the hind leg, tumor growth can be monitored by serial diameter measurements which are converted to tumor weight by means of a predetermined conversion equation.[223]

3. Experimental

3.1 Equipment

Infrared spectra were recorded on a Perkin-Elmer 267 Grating Infrared Spectrophotometer.

Proton nuclear magnetic resonance ('H-NMR) spectrometry was carried out on a Varian EM360A NMR Spectrometer. All chemical shift values are reported in ppm (δ) units and coupling constants (J) are given in Hz.

Chemical ionization mass spectrometry (CIMS) using ammonia was performed by the Department of Chemistry, University of Alberta, using an A.E.I. MS 12 Mass Spectrometer. Electron impact mass spectrometry (EIMS) was determined with either a Hewlett Packard 5995A Gas Chromatograph/Mass Spectrometer system in this faculty or an A.E.I. MS 50 Mass Spectrometer in the Department of Chemistry. Exact mass measurement was used in lieu of elemental analysis and the results are expressed in m/e units.

Two high pressure liquid chromatography systems (HPLC) were used. In system I, a silica gel column (E. Merck Silica Gel 60 Prepacked Size B) was connected to a 2-litre conical flask containing the development solvent (chloroform : methanol 1:1 v/v) which was pressurized with nitrogen gas to produce a solvent flow rate of 5 mL min^{-1}. The column outlet was connected to a Pharmacia UV Monitor ultra-violet (UV) detector (254 nm) and a NaI(Tl) crystal (2 in by 2 in). In system II, a C-18 Bondapak® reverse phase column (3.9 mm

I.D. X 30 cm length, 10 μ particle size, Waters Assoc.) was used in conjunction with a Tracor 995 Isochromatographic Pump and a Pye Unicam variable wavelength (190 - 390 nm) LC3 UV Detector. All solvents used in HPLC were de-gassed for 1 hr prior to HPLC using an in-house vacuum system.

Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected unless stated otherwise.

The micro thin-layer chromatography (TLC) plates used were Whatman MK6F Microslides. These were developed using either solvent system I (chloroform : 1,4-dioxane : methanol 8:6:3 v/v) or solvent system II (chloroform : ethanol 4:1 v/v). Visualization was effected using a short wavelength UV light.

Thermal neutron activation of ^{81}Br to ^{82}Br was performed in the University of Alberta SLOWPOKE Reactor (UASR) at a flux of 1 X 10^{12} n cm^{-2} sec^{-1} for 3 to 4 hr.

Liquid scintillation (LS) counting was carried out using a Beckman LS 9000 counter.

Gamma counting was performed with either a Searle 1185 or a Beckman Gamma 8000 gamma counter.

Analysis of urinary metabolites was carried out using either a Berthold LB2832 Automatic TLC Linear Analyzer equipped with a Berthold LB2821 Proportional Counter and a Canberra Series 40 MCA or a Beckman LS 9000 LS counter.

3.2 Reagents and Solvents

Chloroform (Baker) and methanol (BDH) were purified by fractional distillation and stored in a brown bottle.

1,4-Dioxane (BDH) was refluxed over calcium hydride overnight followed by distillation and storage in a dry brown flask under nitrogen.

Dimethylformamide (DMF, BDH) and pyridine (BDH) were purified by refluxing the solvents overnight with calcium hydride followed by distillation and storage in a brown bottle containing 3Å molecular sieves (Aldrich).

All solids were dried either in a desiccator over phosphorus pentoxide or in an evacuated oven at 100°C.

Silver chloride-cellulose (AgCl-cellulose) was prepared by triturating silver nitrate (2 g) with 98 g of microcrystalline cellulose (Camag). Sufficient water was added to make 500 mL. The mixture was stirred in the dark overnight and then acidified with 2 mL of 6N hydrochloric acid (HCl). The aqueous layer was decanted and the residue washed well with water. The solid was filtered, dried in a rotary evaporator and stored in a dark container.

3.3 Chemistry

3.3.1 6-Chlorouracil, 6-ClU (82a)

6-Chlorouracil,[54] 82a, was prepared from acid hydrolysis of 2,4-dimethoxy-6-chloropyrimidine, 81 (5.1664 g, 0.0296 M, Aldrich) which was refluxed with glacial HOAc (468

mL) and 2N HCl (60 mL) for 1 hr. The solvents were removed under reduced pressure. The residue was recrystallized twice from water to afford colorless needles of 82a (1.23 g, 50% yield); MP: 295 - 300°C, dec., corr., reported: 298 - 300°C;[54] TLC *Rf* (system I): 0.63; ^{1}H-NMR (DMSO-d_6): 5.75 (s, 1H, H-5).

3.3.2 [^{36}Cl]-6-Chlorouracil, [^{36}Cl]-6-ClU (82)

The title compound 82 having a specific activity of 5.66 MBq mM^{-1} was synthetized by D.N. Abrams from [^{36}Cl]-calcium chloride and 83 in DMF.[224]

3.3.3 6-Iodouracil, 6-IU (83)

6-Iodouracil,[54] 83, was synthetized by heating at reflux 6-ClU (0.404 g, 2.75 mM) and sodium iodide (1.648 g, 10.99 mM, Baker) in DMF (4.5 mL) under a nitrogen atmosphere at 150°C for 1 hr. The solvent was then removed *in vacuo*. The dark colored residue was recrystallized from ethanol to give a white powder (0.446 g, 68.35% yield); MP: 278 - 280°C, dec., corr., reported: 279 - 280°C;[54] ^{1}H-NMR (DMSO-d_6): 6.05 (s, 1H, H-5); TLC *Rf* (system II): 0.54; HPLC (system II, 5% aqueous methanol, 2mL min^{-1}): 4.8 min.

3.3.4 [^{82}Br]-Ammonium Bromide, [^{82}Br]-NH_4Br

Two methods of [^{82}Br]-NH_4Br preparation were employed:

1. Method A: From ammonium bromide, (NH_4Br), with natural abundance bromine

Dry ammonium bromide (Fisher Certified A.C.S.) was irradiated in a double plastic vial in the UASR using the $^{81}Br(n, \gamma)^{82}Br$ reaction at a neutron flux of 1×10^{13} n cm^{-2} sec^{-1} for 3 to 4 hr. A typical yield was 0.53 MBq mg^{-1} of NH_4Br for a 3 hr irradiation period. The [^{82}Br]-NH_4Br was used as such after a cooling period of 2 to 4 hr.

2. Method B: From enriched [^{81}Br]-NH_4Br

Enriched [^{81}Br]-NH_4Br was also used in the preparation of [^{82}Br]-NH_4Br. A solution of [^{81}Br]-NaBr (100 mg, 97.81% enriched in ^{81}Br, Oak Ridge National Laboratory) in deionized distilled water (1 mL) was added to a column containing 50 g Amberlite IR-120 cation (ammonium) exchange resin (Mallinckrodt Chem. Works). Elution with deionized distilled water (25 mL) followed by removal of the solvent *in vacuo* afforded [^{81}Br]-NH_4Br in quantitative yield. Tritrimetric analysis[233] of the product for ammonium and bromide indicated an ammonium : bromide ratio of 0.8:1. Neutron activation analysis indicated the sodium line of 2754 KeV to be less than 1% of the total activity. The [^{81}Br]-NH_4Br was used without further purification. Thermal neutron irradiation of the enriched [^{81}Br]-NH_4Br was carried out similarily to the procedure described above.

3.3.5 6-Bromouracil, 6-BrU (85a)

A solution of 6-IU (74.2 mg, 0.31 mM) in DMF (3 mL) was heated with NH_4Br (153 mg, 1.56 mM) at 150°C for 1 hr. Recrystallization from methanol afforded 6-BrU (41.8 mg, 70% yield). The colorless needles did not melt below 280°C; TLC *Rf* (system I): 0.67, (system II): 0.34; HPLC (system II, 5% aqueous methanol, 2 mL min^{-1}): 3.3 min; ^{1}H-NMR (DMSO-d_6): 5.8 (s, 1H, H-5); EIMS (high resolution MS): Exact mass calculated for $C_4H_3N_2O_2{}^{79}Br$: 189.9979, found: 189.9380, 28.45%; Exact mass calculated for $C_4H_3N_2O_2{}^{81}Br$: 191.9959, found: 191.9359, 27.60%.

3.3.6 [^{82}Br]-6-Bromouracil, [^{82}Br]-6-BrU (85)

Two synthetic methods were employed:

1. Reaction with [^{82}Br]-NH_4Br

   [^{82}Br]-6-Bromouracil (85) was produced by a bromine-for-iodine exchange reaction using 6-IU (5 mg, 0.022 mM) and [^{82}Br]-NH_4Br (prepared from [^{81}Br]-enriched NH_4Br, 7.6 mg, 0.08 mM, 3 hr irradiation at a flux of 1 X 10^{13} n cm^{-2} sec^{-1}) at 150°C for 1 hr. Purification by micro-TLC using solvent system II afforded [^{82}Br]-6-BrU (2.8 mg, 99.36% chemical yield, 26.95% radiochemical yield). The product was compared with **85a** by means of micro-TLC in solvent systems I and II and by HPLC (system II, 5% aqueous methanol, 2 mL min^{-1}) and was found to be identical in all respects. The specific activity was determined to be 68.40 MBq mM^{-1} as measured

by combined UV absorbance at 260 nm and LS counting in Aquasol II®.

2. Direct neutron irradiation of 6-BrU

Natural abundance dry 6-BrU was irradiated in the UASR as described for NH_4Br. Typical recoverable radioactivity associated with 6-BrU was 3 to 6% of the total radioactivity produced.

3.3.7 2,2'-Anhydro-1-β-D-arabinofuranosyluracil (87)

The title compound, 87, was prepared according to the method of Hampton and Nichol.[155] A mixture of uridine (0.502 g, 2.05 mM), diphenylcarbonate (0.572 g, 2.67 mM) and sodium bicarbonate (14.1 mg) in DMF (1 mL) was heated to 150°C for 30 min. The reaction mixture was poured onto 20 mL diethyl ether. The gummy residue was recrystallized twice from methyl hydrate to give compound 87 (0.271 g, 58.43% yield); MP 236 - 238°C, reported: 238 - 244°C;[155] TLC *Rf* (system I): 0.17; HPLC (system I): 24 min.

3.3.8 1-(2'-Bromo-2'-deoxy-β-D-ribofuranosyl)uracil, 2'-BrUdR (88a)

Four methods were employed in the synthesis of 2'-BrUdR:

1. Reaction with hydrogen bromide[72]

Compound 87, (57.4 mg, 0.25 mM) was dissolved in 3 mL of dry trifluoroacetic acid which was previously saturated with anhydrous hydrogen bromide at 0°C. The

flask was sealed and the contents were stirred for 4 days at room temperature. The reaction mixture was concentrated under reduced pressure to give a dark brown syrup which was washed repeatedly with light petroleum ether. Recrystallization from ethanol - light petroleum ether afforded compound **88a** (45.2 mg, 58% yield); MP: 188 - 189°C, reported: 186 - 190°C;[72] TLC *Rf* (system I): 0.6; HPLC (system I): 18 min; IR (KBr, 0.5%) 605 cm^{-1} m, C-Br;[223] ^{1}H-NMR (D_2O): 6.0 (d, J = 5.5 Hz, 1H, H-5); 7.9 (d, J = 8 Hz, 1H, H-6); 6.3 (m, 1H, H-1'); 4.7 (m, 1H, H-2'); CIMS(NH_3): Mass calculated for $C_9H_{11}N_2O_5{}^{79}Br$ + 1: 307, found: 307, 2.25%; Mass calculated for $C_9H_{11}N_2O_5{}^{81}Br$ + 1: 309, found: 309, 2.25%

2. Reaction with ammonium bromide

A solution of **87** (51.2 mg, 0.227 mM), NH_4Br (50.6 mg, 0.516 mM) and *p*-toluenesulfonic acid (39.1 mg, 0.227 mM) in dry DMF (5 mL) was heated at 100°C for 4 hr. The solvent was removed *in vacuo*. Acetone (20 mL) was added to the residue and the insoluble material was filtered. Evaporation of the acetone *in vacuo* yielded a syrupy liquid to which 5 mL of water was added. The aqueous solution was washed with three 10 mL portions of benzene. Evaporation of the water *in vacuo* followed by preparative HPLC (system I) afforded **88a** (62% yield) which exhibited an elution time of 18 min. Removal of the solvent produced small colorless needles which were identical (MP, IR, ^{1}H-NMR and MS) with an authentic

sample prepared by Method 1.

3. Reaction with sodium bromide

Reaction of compound 87 (77.9 mg, 0.34 mM) with sodium bromide (191 mg, 1.86 mM) and *p*-toluenesulfonic acid (58.5 mg, 0.34 mM) in dry DMF (5 mL) as described previously afforded a light brown solution. Analysis by HPLC (system I) indicated a chemical yield of 80%.

4. Reaction with lithium bromide

Reaction of 87 (45.6 mg, 2 mM) with lithium bromide (34.7 mg, 0.4 mM) and *p*-toluenesulfonic acid (34.4 mg, 0.2 mM) in dry DMF (5 mL) afforded 88a in 70% yield as determined by HPLC analysis (system I).

3.3.9 [^{82}Br]-1-(2'-Bromo-2'-deoxy-β-D-ribofuranosyl)uracil, [^{82}Br]-2'-BrUdR (88)

In the laboratory [^{82}Br]-2'-BrUdR was prepared by two methods:

1. Reaction with [^{82}Br]-NH_4Br

A solution of 87 (3.6 mg, 0.016 mM), [^{82}Br]-NH_4Br (from natural abundance NH_4Br, 18 mg, 0.18 mM, 3 hr of irradiation in the UASR) and *p*-toluenesulfonic acid (2.7 mg, 0.018 mM) in dry DMF (0.2 mL) was heated at 100°C for 6.5 hr. After cooling in an ice bath the solution was applied to one 20 X 20 cm silica gel DSF-5 plate 1.0 mm in thickness which was developed using solvent system I. Extraction of the fraction corresponding to an authentic sample of 88a with warm methanol and quanti-

tation by UV spectrometry at 260 nm indicated a 73.4% chemical yield of [^{82}Br]-2'-BrUdR (3.6 mg) with a specific activity of 21.53 MBq mM^{-1} corrected to the end of bombardment (EOB) as determined by LS counting (6.3% radiochemical yield).

2. Direct irradiation of 2'-BrUdR

A sample of 2'-BrUdR (2 mg) containing natural abundance bromine was irradiated for 3 hr in the UASR. The sample was dissolved in 1 mL of methanol, after standing for 24 hr to allow ^{80m}Br to decay prior to HPLC purification (system I). Twenty-five percent of the radioactivity produced was recovered and could be associated with 2'-BrUdR which had a measured specific activity of 22.2 MBq mM^{-1} corrected to the EOB as indicated by LS counting.

3.3.10 1-(2',3',5'-Tri-O-methylsulfonyl-β-D-ribofuranosyl)-uracil, (64)

To a solution of of uridine (10 g, 0.041 M) in dry pyridine (100 mL) at 0°C dry methylsulfonyl chloride (15.6 g, 0.14 M) was added with stirring.[223] The reaction mixture was allowed to stand for 3 hr at 0°C and then poured onto 3 L of ice-water. This mixture was stirred for 1 hr. The pale yellow precipitate was filtered off, washed three times with ethanol (10 mL) and three times with ether (10 mL). Recrystallization from 50% ethanol afforded the title compound (64) as colorless needles (14.55 g, 75% yield); MP:

178 - 180°C, dec., corr., reported: 181 - 182°C, dec.;[228] TLC *Rf* (system I): 0.35; ^{1}H-NMR (DMSO-d_6): 3.40 (s, 9H, CH_3).

3.3.11 2,2'-Anhydro-1-(5'-O-benzoyl-3'-O-methylsulfonyl-β-D-arabinosyl)uracil (65)

To a solution of sodium benzoate (10 g, 0.0692 M) in acetamide (86 g, 1.4560 M) at 100°C compound **64** (5 g, 0.0104 M) was added with stirring and the temperature was elevated to 115°C for 35 min.[228] The yellow solution was poured onto ice-water (750 mL) and allowed to stand for 1 hr in a refrigerator at 10°C. Recrystallization of the white precipitate from ethanol : water (1:1 V/V) afforded the title compound **65** as colorless needles (3.5269 g, 82.64% yield); MP: 238 - 239°C, dec., corr., reported: 222 - 226°C;[228] TLC *Rf* (system I): 0.58, (system II): 0.25; ^{1}H-NMR (DMSO-d_6) 3.45 (s, 3H, CH_3); 8.10 (m, 5H, Ph).

3.3.12 1-(5'-O-Benzoyl-3'-O-methylsulfonyl-β-D-arabinosyl)-uracil (66)

Concentrated HCl (2.4 mL) was added slowly to a suspension of compound **65** (0.5986 g, 1.47 mM) in an acetone : water mixture (180 mL, 1:1 v/v). The mixture was stirred for 24 hr to achieve complete dissolution.[184] The reaction volume was reduced by half *in vacuo* to afford the title compound, **66**, as colorless needles which were collected, washed well with ice-water and dried. The yield was 0.594 g

(99.23%); MP: 80 - 84°C, corr., reported: 81 - 84°C;[184] TLC *Rf* (system II): 0.71; ^{1}H-NMR (DMSO-d_6): 3.45 (s, 3H, CH_3); 8.10 (m, 5H, Ph).

3.3.13 1-(5'-O-Benzoyl-2',3'-epoxy-β-D-lyxofuranosyl)uracil, (67)

Compound **66** (672 mg, 1.58 mM) was added slowly to 50 mL of 1N NH_4OH.[184] A colorless precipitate was formed after 70 min of stirring. The pH of of the solution was adjusted, after stirring for a further 20 min, with glacial HOAc to pH 6 - 7 and the mixture was cooled in an ice bath. The precipitate was collected and washed well with ice-water. Recrystallization from ethanol afforded the title compound **67** (colorless plates, 333.5 mg, 64.67% yield); MP: 187 - 190°C, corr.; reported: 188.5 - 190°C;[184] TLC *Rf* (system I): 0.82, (system II): 0.92; ^{1}H-NMR (DMSO-d_6): 4.52 (m, 3H, H-2', H-3', H-4'); 8.10 (m, 5H, Ph).

3.3.14 1-(2',3'-Epoxy-β-D-lyxofuranosyl)uracil, (89)

Compound **67** (77.2 mg, 0.2339 mM) was dissolved in 0.5N NaOH (1.2 mL) and stirred for 50 min at room temperature.[184] The solution was divided into two portions, each treated with 750 mg of Dowex 50W-X8® (H^+, 20 - 50 mesh, Baker) and then filtered. The combined filtrate was extracted thrice with ether (40 mL). The aqueous layer was taken to dryness *in vacuo*. Ethanol (5 mL) was added and removed under reduced pressure. Purification using preparative TLC (20 X 20 cm

silica gel DSF-5 plate 1.0 mm in thickness, solvent system II) afforded **89** as a colorless powder (47.1 mg, 89% yield); MP: 138 - 140°C, corr., reported: 139.5 - 140.5°C;[184] TLC *Rf* (system II): 0.7.

3.3.15 1-(3'-Chloro-3'-deoxy-β-D-arabinofuranosyl)uracil, 3'-ClUdR (90a)

A solution of **89** (17.2 mg, 0.0761 mM) in 5% HCl (0.1 mL) was heated at 90°C for 1 hr.[223] Purification by micro-TLC (system II) followed by recrystalliztion from methanol gave 11 mg of the title compound **90a** (55%); MP: 158 - 159°C, corr., reported: 159 - 160°C;[223] TLC *Rf* (system II): 0.8; EIMS (high resolution MS): Exact mass calculated for $C_9H_{11}N_2O_5{}^{35}Cl$: 262.05, found: 262.05, 1.1%; Exact mass calculated for $C_9H_{11}N_2O_5{}^{37}Cl$: 264.10; found: 264.05, 0.4%.

3.3.16 [^{36}Cl]-1-(3'-Chloro-3'-deoxy-β-D-arabinofuranosyl)-uracil, [^{36}Cl]-3'-ClUdR (90)

[^{36}Cl]-Hydrochloric acid (2.48N, 99.0% radionuclidic purity, 99.0% radiochemical purity) with a specific activity of 5.82 MBq mM^{-1} was purchased form NEN. Compound **89** (56.7 mg, 0.2509 mM) was dissolved in 0.071 mL of distilled water to which 0.099 mL of 2.48N [^{36}Cl]-HCl had been added. The mixture was heated at 90° for 1 hr, passed through a 2 cm column of cellulose-AgCl and then concentrated *in vacuo*. Purification was performed on micro-TLC using solvent system II. The appropriate band was extracted with 20% methanol in

chloroform. The product was obtained as colorless needles after recrystallization from methanol (32.3 mg, 49% chemical yield). The specific activity was determined to be 5.48 MBq mM^{-1}.

3.3.17 1-(3'-Bromo-3'-deoxy-β-D-arabinofuranosyl)uracil, 3'-BrUdR (91a)

3'-BrUdR (91a) was synthesized by reacting the 2',3'-lyxoepoxide 89 (1.1 mg, 0.0049 mM) with NH_4Br (1.2 mg, 0.0122 mM) in 0.25 mL ethanol. The reaction vial containing the reactants was placed in a heating block at 150°C. The cap of the vial was loosened slightly after 15 min to facilitate venting of the ethanol solvent. Analytical HPLC (system II, 5% aqueous methanol, 1 ml min^{-1}) after a 60 min reaction time indicated a chemical yield of 67.14% (1.0 mg); MP: 185 - 186°C, corr., reported: 185.0 - 185.5°C;[111] TLC *Rf* (system II): 0.86; ^{1}H-NMR (DMSO-d_6): 5.6 (d, J = 8 Hz, 1H, H-5); 7.7 (d, J = 8 Hz, 1H, H-6); 6.2 (d, J = 6 Hz, 1H, H-1); 4.58 (m, 1H, H-2'); 4.2 (m, 2H, H-3', H-4'): CIMS (NH_3): Mass calculated for $C_9H_{11}N_2O_5{}^{79}Br$ + 18: 324, found: 324; Mass calculated for $H_{11}N_2O_5{}^{81}Br$ + 18: 326, found: 326, 16.90%.

3.3.18 [^{82}Br]-1-(3'-Bromo-3'-deoxy-β-D-arabinofuranosyl)-uracil, [^{82}Br]-3'-BrUdR (91)

The title compound can be synthesized by two methods:

1. Reaction with [^{82}Br]-NH_4Br

[^{82}Br]-3'-BrUdR was synthesized as described for **91a** from **89** (2.1 mg, 0.009 mM) and **84** prepared from NH_4Br with natural abundance bromine (1.75 mg, 0.018 mM) in ethanol (0.5 mL) at 150°C for 1 hr in 67.1% chemical yield (2.0 mg) as indicated by HPLC (system II). The radiochemical yield was 33.57% and the specific activity was determined to be 28.99 MBq mM^{-1}.

2. Direct neutron activation of 3'-BrUdR in the UASR

Direct activation of **91a** in the UASR followed by HPLC analysis (system II, 5% aqueous methanol, 1 mL min^{-1}) resulted in a 10 to 15% recovery of the radioactivity produced. The specific activity was determined to be 8.99 MBq mM^{-1}.

0.2 Preparation of Tumor Bearing Animals

Mice, 20 to 25 g, were purchased from the University of Alberta Health Sciences Small Animal Center. Male BDF$_1$ mice were used throughout the project except for [^{34}Cl]-3'-ClUdR where female animals were substituted. All animals bore Lewis Lungs tumor[230] except where noted.

The tumor line was maintained by serial transplantation every two weeks. The tumor donor-mouse was sacrificed by cervical dislocation and immersed briefly in 70% alcohol for disinfection followed by washing under cold running water. The tumor nodule was surgically exposed and a portion of the tumor surface was removed and immersed immediately in normal

saline in a petri dish. The necrotic central cell mass was scraped off with the blunt tips of a pair of forceps leaving only the firm outer periphery of cells which was transported to another petri dish containing normal saline and diced into small squares measuring approximately 2 mm^3. The prepared tumor pieces were injected subcutaneously into the right flank region midpoint between the fore and the hind limb of the recipient mice using a 14-gauge needle and a trochar.

3.5 Preparation for Injection

The compounds under study were prepared as described in the chemistry section. The reaction mixture was purified by micro-TLC. Extraction of the band corresponding to the *Rf* of an authentic sample with 20% methanol in chloroform and passage through a 2 cm column of AgCl-cellulose previously washed with the extracting solvent. The solvent was removed *in vacuo* and the residue was reconstituted in normal saline, analyzed for chemical and radiochemical purity by combined TLC and radioactivity counting. The sample was ready for intravenous bolus injection into the tail vein after passage through a 10 μ Millipore® filter (Millipore Corp.). The injected chemical dose was always 0.1 mg/mouse or less and present in 0.1 mL of normal saline.

3.6 Tissue Distribution

Entire organs were removed for counting except where noted following euthanasia with ether. Blood was sampled by cardiac puncture after opening of the abdominal wall and diaphragm. All stomach and GIT samples were emptied of their food contents. The lungs were blotted to remove surface blood contamination. The heart was cut open and cleared of blood clots in the chambers. An area of the skin devoid of blood capillaries was removed from the left flank opposite to the tumor side. Muscle was sampled from the left thigh. The left tibia and fibula were removed as representative samples of the long bone.

Sample preparation and LS counting of ^{36}Cl requires the use of a small sample size. The total mass of the organs was noted but only the left kidney, tip of the left lobe of the liver, 1 cm section of the duodenum and the left testicle were used in the actual counting of radioactivity.

3.7 Liquid Scintillation Counting of Chlorine-36

Distilled water (0.1 mL) Protosol® (2 mL, NEN) were added to samples in 20 mL glass LS vials. Solubilization of the tissues was accelerated by immersing the vials in a hot water bath at 50°C overnight. Color quenching was reduced by the addition of 30% hydrogen peroxide (0.2 to 1.5 mL) to samples of blood, spleen, skin, kidney, lungs, heart and tumor. Excess peroxide was decomposed by warming in a water bath at 50°C. Aquasol II® (10 mL, NEN) was added to each

sample followed by 0.1 mL of 4% stannous chloride in glacial HOAc.

Dark adaptation for 24 hr preceeded initiation of the counting process. All samples were counted using a Beckman LS 9000 Liquid Scintillation Counter. A quench correction curve was prepared for automatic dpm calculation by the H number method. A series of 10 standards each containing Aquasol II® (10 mL) and [^{3}'Cl]-6-ClU (190,560 dpm) were prepared. One vial was reserved as an unquenched standard and to the others was added varying amounts of solubilized tissue preparation. The standards together with three background samples were counted using the following counting parameters: Beckman LS 9000, lower level 300, upper level 850, preset counting time 10 min, preset error 1%.

3.8 Gamma Counting of Bromine-82

Samples were counted in 25 X 80 mm glass gamma vials (Amersham Corp.) using either a Searle 1185 or a Beckman Gamma 8000 gamma-counter. The channel settings were selected to allow low background, low level counting. Counting did not begin until 40 hr had elapsed after the EOB to minimize contribution from the shorter-lived bromine isotopes to the overall count rate. The settings were as follows: Searle 1185, maximum energy range 2 Mev, lower channel setting 200, upper channel setting 750, preset counting time 10 min, preset counts 40K; Beckman Gamma 9000, energy range 1 MeV, lower level 500, upper level 1000, vial position low, preset

time 10 min, preset error 1%.

3.9 Whole-body Elimination Studies

The whole-body elimination of [^{82}Br]-2'-BrUdR and [^{82}Br]-6-BrU was studied using normal male BDF$_1$ mice. Total body radioactivity was measured at various predetermined times in a whole-body counter. The instruments and their settings were: NaI(Tl) crystal 3 in X 3 in, Ortec Model 486 Amplifier and Pulse Height Analyser, Canberra Nuclear Counter Model 1775, Canberra H.V. Power Supply Model 3002, Canberra CI Model 1400, energy range 2 MeV, coarse gain 8, fine gain 8.5, high voltage 1050, lower level 250, upper level 750, preset time 1 min.

Since whole-body counting of animals injected with ^{34}Cl is impractical, elimination analysis was performed by LS counting of radioactivity in whole-blood. Blood samples were removed from the animals and processed for counting as described.

3.10 Analysis of Urinary Samples

Urinary samples were analysed using either a Beckman LS 9000 or a Berthold TLC Linear Analyzer. Urinary samples (0.001 mL) were spotted on micro-TLC plates and developed using solvent system II. The spots were marked after visualization using a short wavelength UV lamp and scraped off the plates. The area between spots were sectioned into approximately 5 mm strips which were also scraped off into

glass LS vials. Distilled water (2 mL) and Aquasol-II® (10 mL) were added to form a gel which was counted in the Beckman LS 9000 after dark adaptation. For analysis using the Berthold TLC Linear Analyzer the urine samples were spotted on micro-TLC plates which were developed in solvent system II for 6 cm. The dry plates were analyzed using the following conditions: analyzer gas 9.94% methane in argon, flow rate 30 mL min^{-1}, high voltage 1555, gain 4, preset time 600 sec.

4. Results and Discussion

4.1 [^{34}Cl]-6-Chlorouracil (82)

4.1.1 Tissuse Distribution

The differential tissue distribution data for [^{34}Cl]-6-ClU in male BDF$_1$ mice bearing subcutaneous Lewis Lung carcinomas are presented in Fig. 3.1 to Fig. 3.4. Neither [^{34}Cl]-6-ClU nor its label was taken up to any significant extent by the tissues studied. Each data point is the mean of five experiments and the vertical bars represent one standard deviation (S.D.).

The tumor is the organ of interest. Muscle is important because of its contribution to background radiation. Long bone is representative of organs of high mitotic index. Their respective tissue:blood ratios are presented in Fig. 3.1. At the 0.25 hr time period, which was the earliest sampling time, the tumor had a higher ratio (0.39) than those of muscle (0.15) and long bone (0.16). All three tissues exhibited a slow but steady rise in tissue:blood ratios with time. The maximum ratio for the tumor was 0.91 at 2 hr which then fell off slightly to 0.85 at 3 hr. Muscle showed a maximum ratio of 1.31 at 2 hr with very large S.D. and the ratio decreased to 0.32 at 3 hr. The uptake of radioactivity by the long bone followed very closely that of the muscle starting at a ratio of 0.16 at 0.25 hr and was elevated slowly to a maximum of 0.47 at 3 hr.

The % dose g^{-1} tissue data for the three tissues and blood are tabulated in Table 6.1. The blood sustained the highest radioactivity level for 2 hr. At the first sampling time of 0.25 hr, 1 g of blood and tumor contained 10.86 and 4.24% of the injected dose respectively. The radioactivity in the blood fell off sharply and at 2 hr represented only 1.78% of the injected dose and at 3 hr 0.83%. Corresponding figures for tumor were 1.27 and 0.64%.

The percent uptake g^{-1} muscle was almost indistinguishable from that of the long bone. At time 0.25 hr they represented 1.58 and 1.69% of the injected dose and were 0.25 and 0.32% respectively at 3 hr.

Other tissues of high mitotic index are GIT, spleen, stomach and skin. The tissue:blood ratios for the GIT and spleen are presented in Table 6.2.

Both GIT and spleen showed very similar values in their tissue:blood ratios and only the former is illustrated in Fig. 3.2 together with stomach and skin. GIT and stomach possessed about the same radioactivity levels from 0.25 to 1 hr. At 2 and 3 hr the stomach showed a greater concentration of radioactivity but not without a large S.D.

The skin appeared to be the most active tissue in utilizing [$^{3'}$Cl]-6-ClU or its label. At 0.25 hr the tissue:blood ratio was 0.37 and at 3 hr the maximum value of 1.32 was reached.

A comparison of the % dose g^{-1} data is given in Table 6.3. The skin accumulated the most radioactivity among the

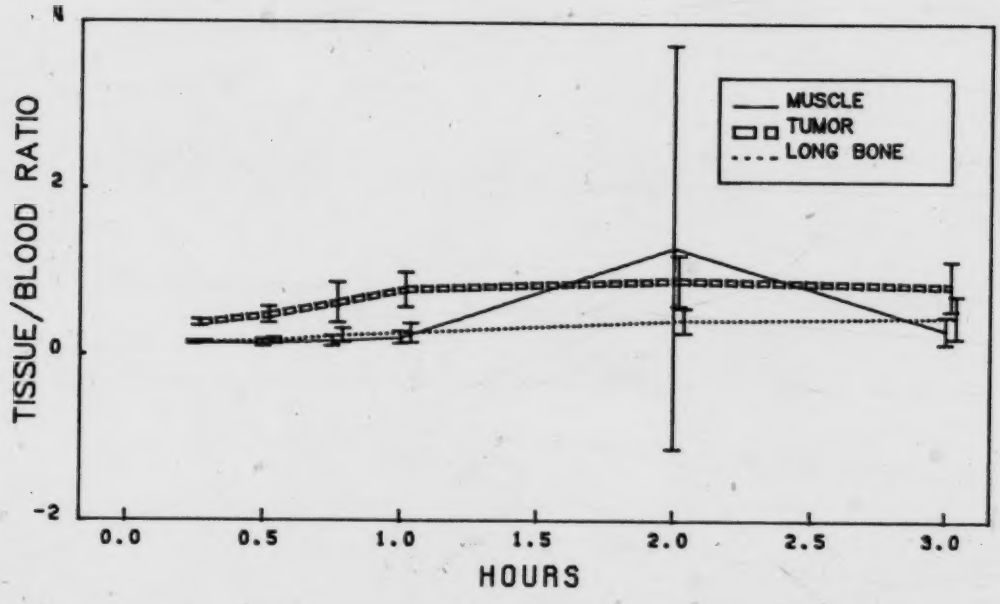


Fig. 3.1: Mean tissue:blood ratios ± 1 S.D. of muscle, tumor and long bone for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

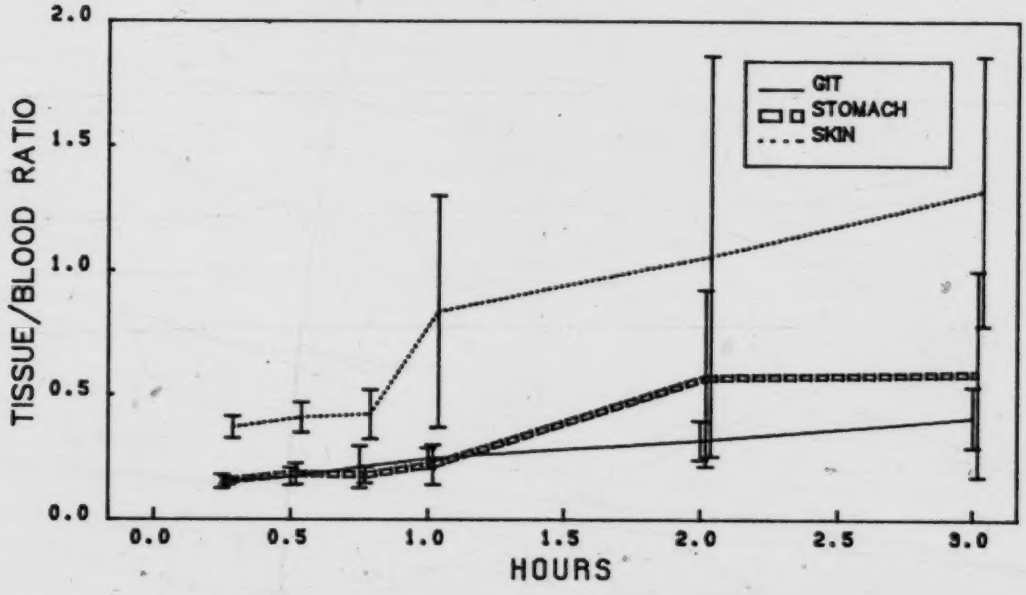


Fig. 3.2: Mean tissue:blood ratios ± 1 S.D. of GIT, stomach and skin for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

Table 6.1: Radioactivity (% dose g^{-1} tissue) in blood, muscle, tumor and long bone for male BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{36}Cl]-6-ClU. n = 5.

	Time (hr)					
	0.25	0.5	0.75	1	2	3
Blood						
% Dose	10.86	7.18	5.60	3.49	1.78	0.83
S.D.	2.06	1.14	3.03	1.19	1.46	0.42
% S.D.	18	15	54	34	82	51
Muscle						
% Dose	1.58	1.08	0.94	0.72	0.87	0.25
S.D.	0.35	0.47	0.60	0.23	1.02	0.18
% S.D.	22	43	63	32	117	70
Tumor						
% Dose	4.24	3.43	3.32	2.72	1.27	0.64
S.D.	1.20	0.78	1.68	0.96	0.66	0.23
% S.D.	28	22	50	35	51	36
Long Bone						
% Dose	1.69	1.22	1.17	0.85	0.62	0.32
S.D.	0.41	0.26	0.36	0.14	0.41	0.08
% S.D.	24	21	30	16	66	25

four organs. At 0.25 hr the percentage was 4.08 and at 3 hr 0.93%.

The gall bladder, kidneys and liver are the major metabolic and/or excretory ogans in the body. Radioactivity levels in these organs often reflect the metabolic fate of labelled exogenous compounds. As illustrated in Fig. 3.3 the gall bladder had very high tissue:blood ratios at 2 hr (8.46) and 3 hr (3.53) both of which were associated with very large S.D. The ratios for the kidneys remained low, yet

Table 6.2: Tissue:blood ratios ± 1 S.D. for GIT and spleen in male BDF_1 mice bearing Lewis Lung carcinomas after an iv injection of [^{36}Cl]-6-ClU. n = 5.

	Time (hr)					
	0.25	0.5	0.75	1	2	3
GIT	0.15 ±0.03	0.18 ±0.04	0.21 ±0.08	0.25 ±0.04	0.32 ±0.08	0.41 ±0.01
Spleen	0.17 ±0.01	0.18 ±0.07	0.18 ±0.05	0.18 ±0.02	0.30 ±0.09	0.35 ±0.13

higher than those for the liver during the first hr. At 2 hr the kidney:blood ratio was 1.73 which declined to 0.41 after 3 hr. The liver:blood ratio went from 0.27 at 1 hr to 0.41 at 2 hr and remained at a similar level of 0.44 at 3 hr.

The % dose g^{-1} figures are given in Table 6.4. These large values must be examined with reservation. The apparently high level of radioactivity g^{-1} in the gall bladder was probably due to the small mass of the organ. The mean organ mass was small (mean = 7.65 mg ± 3.97, range = 3.15 mg, n = 30). The % dose per organ (gall bladder) would provide better perspectives of radioactivity uptake by this organ (Table 6.5).

The data suggest a small biliary uptake of injected radioactivity the nature of which was not determined, but could include the unmetabolised compound and/or its metabolites. The amount of radioactivity in the kidneys and liver

Table 6.3: Radioactivity (% dose g^{-1} tissue) in GIT, spleen, stomach and skin for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

	Time (hr)					
	0.25	0.5	0.75	1	2	3
GIT						
% Dose	1.69	1.26	1.11	0.85	0.58	0.32
S.D.	0.49	0.34	0.51	0.28	0.53	0.14
% S.D.	28	26	46	33	92	42
Spleen						
% Dose	1.87	1.28	0.94	0.62	0.44	0.26
S.D.	0.39	0.51	0.50	0.14	0.31	0.10
% S.D.	20	40	53	23	69	38
Stomach						
% Dose	1.71	1.33	0.94	0.77	0.67	0.40
S.D.	0.36	0.36	0.36	0.34	0.24	0.21
% S.D.	20	26	38	44	35	52
Skin						
% Dose	4.08	2.95	2.20	2.68	1.27	0.93
S.D.	1.11	0.71	0.88	1.30	0.67	0.21
% S.D.	27	24	39	48	52	22

was high at 0.25 hr (7.54 and 2.69% of the injected dose respectively) and decreased rapidly with time.

The lungs and heart possess little or no metabolic or excretory function for pyrimidines. The tissue:blood ratios for the lungs remained almost constant throughout with observed values of 0.44 at 0.25 hr and 0.51 at 3 hr (Fig. 3.4). A similar tendency was also shown by the heart with corresponding ratios of 0.27 and 0.33 (Fig. 3.4).

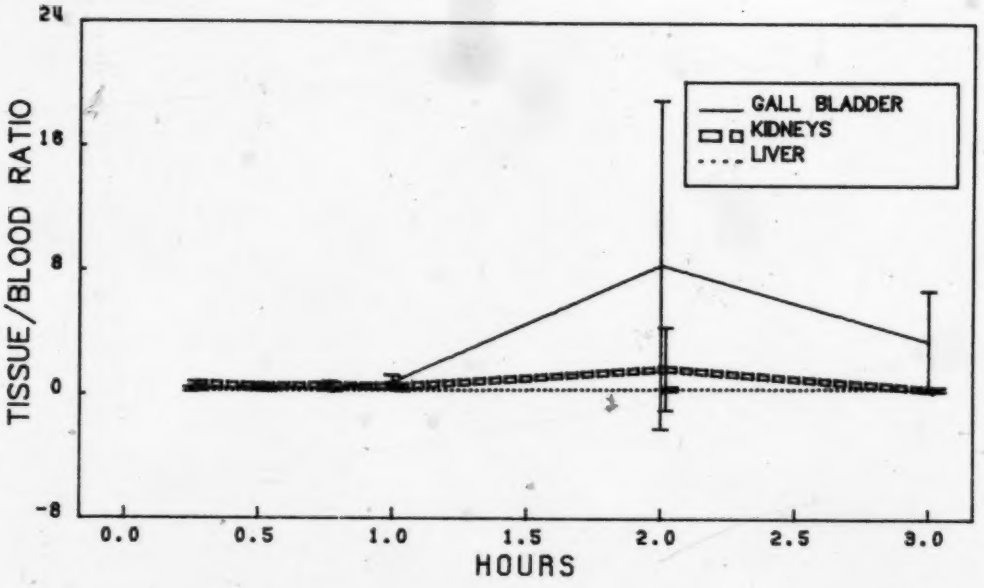


Fig. 3.3: Mean tissue:blood ratios of gall bladder, kidneys and liver for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

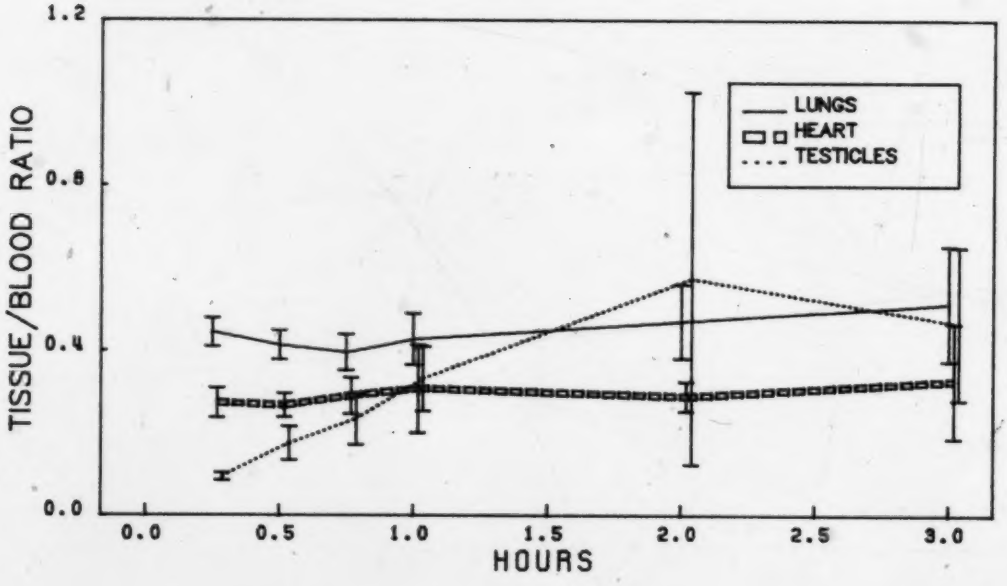


Fig. 3.4: Mean tissue:blood ratios of lungs, heart and testicles for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

Table 6.4: Radioactivity (% dose g^{-1} tissue) in gall bladder, kidneys and liver for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

	Time (hr)					
	0.25	0.5	0.75	1	2	3
Gall bladder						
% Dose	3.33	3.86	2.03	3.12	5.74	1.92
S.D.	1.69	1.74	0.44	2.63	5.80	0.94
% S.D.	50	44	21	84	101	48
Kidneys						
% Dose	7.54	3.69	3.00	1.82	5.11	0.32
S.D.	2.35	0.72	1.19	0.48	9.93	0.15
% S.D.	31	19	39	26	194	46
Liver						
% Dose	2.69	1.52	1.36	0.94	0.66	0.36
S.D.	0.67	0.31	0.63	0.32	0.58	0.26
% S.D.	25	20	46	34	88	70

Table 6.5: % Dose per gall bladder after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

	0.25 hr	0.50 hr	0.75 hr	1 hr	2 hr	3 hr
Mean	0.019	0.018	0.007	0.014	0.025	0.013
S.D.	0.007	0.007	0.005	0.005	0.011	0.004
% S.D.	38	37	27	31	44	28

Testicles contain germinal centers of high cellular turnover rate. However only a moderate increase in radioactivity could be observed in this organ with ratios from 0.01 at 0.25 hr to 0.47 at 3 hr (Fig. 3.4).

The percentages of dose retained by 1 g of lungs, heart testicles are listed in Table 6.6. The radioactivity levels of the lungs were consistently higher than those of the other two organs.

Table 6.6: Radioactivity (% dose g^{-1} tissue) in lungs, heart and testicles for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{123}I]-6-IUdR. n = 5.

	Time (hr)					
	0.25	0.5	0.75	1	2	3
Lungs						
% Dose	4.83	2.95	2.13	1.46	0.75	0.39
S.D.	0.95	0.41	0.94	0.39	0.55	0.14
% S.D.	19	14	44	26	72	37
Heart						
% Dose	2.93	1.91	1.60	0.99	0.50	0.24
S.D.	0.39	0.28	0.83	0.14	0.40	0.08
% S.D.	13	14	52	14	79	32
Testicles						
% Dose	1.04	1.27	1.21	1.12	0.71	0.34
S.D.	0.23	0.40	0.49	0.33	0.43	0.10
% S.D.	21	31	40	29	60	28

4.1.2 Whole-body Clearance of Radioactivity

Analysis of blood clearance data using the U of A MTS *Nonlin* curve fitting program generated a bi-exponential curve as shown in Fig. 3.5 with the four excretion parameters listed in Table 6.7.

Component I and II represent the long-lived and short-lived functions with half-lives of 1.32 and 0.31 hr and 4.30 and 12.42% of injected radioactivity g^{-1} blood (25.74 and 74.26% of blood radioactivity) of the injected dose respectively.

4.1.3 Analysis of Urinary Samples

Urinary samples were analysed as described in the experimental section. Of the 15 samples taken, 8 contained only one radioactive compound (Table 6.8). A second radioactive metabolite was observed in the remaining 7 samples. The second metabolite can be described as minor with concentrations varying from 7.44 to 26.44% of the total urinary radioactivity. It is also more polar than the first compound with a *Rf* value less than 0.1 (solvent system II) and was tentatively identified as ^{3}'Cl^{-} by co-chromatography with [^{3}'Cl]-NaCl.

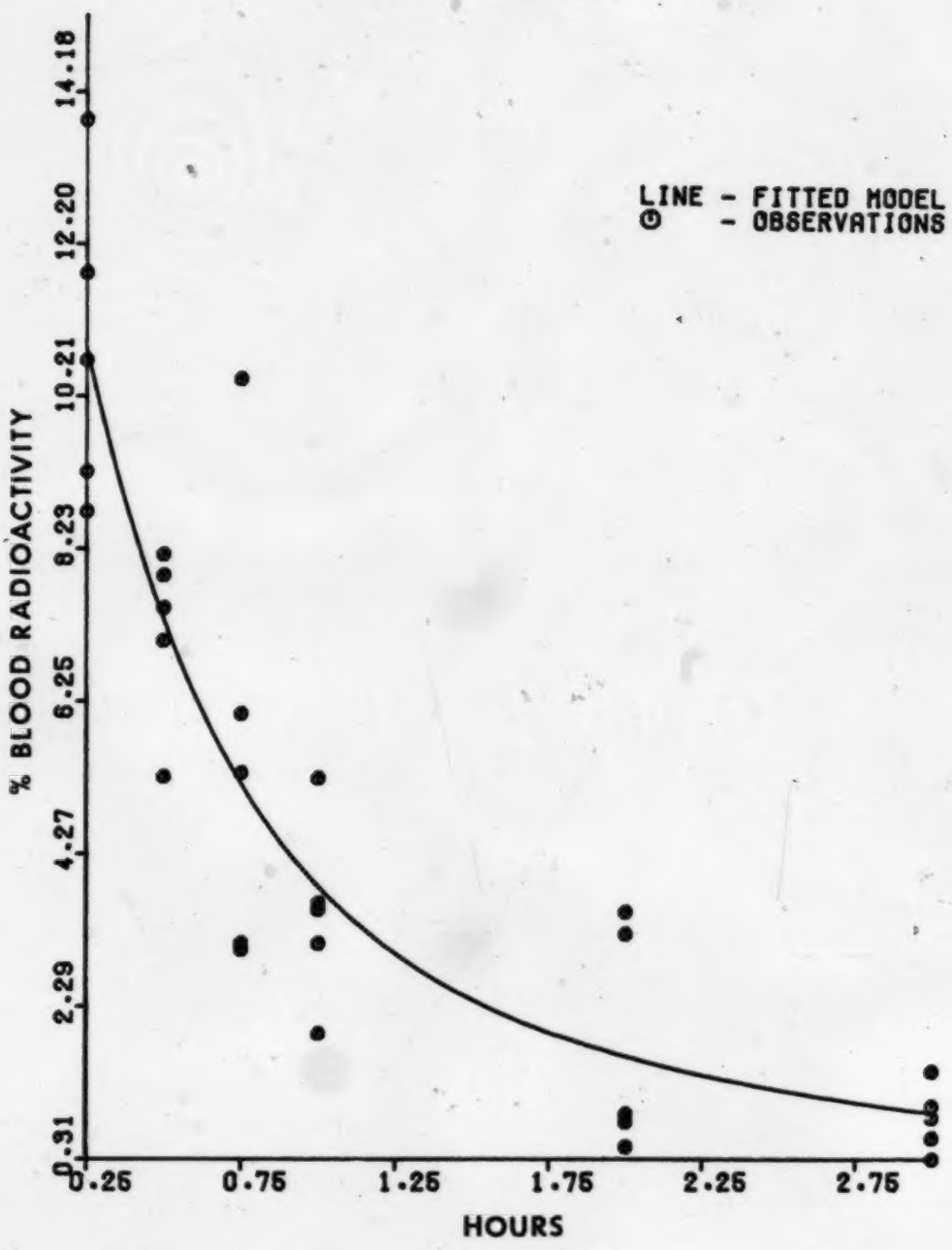


Fig. 3.5: Clearance of blood radioactivity in BDF$_1$ male mice after an iv injection of [^{3}'Cl]-6-ClU. n = 5.

Table 6.7: Blood clearance parameters for male BDF, mice mice bearing Lewis Lung carcinomas after an iv injection of [^{34}Cl]-6-ClU. n = 5.

	Component I	Component II
% Dose	4.30 ± 13.30	12.42 ± 10.27
Half-life (hr)	1.32 ± 2.94	0.31 ± 0.38

Table 6.8: Urinary metabolites of [^{34}Cl]-6-ClU expressed as percentages of urinary radioactivity.

Time (hr)	Animal	[^{34}Cl]-6-ClU	^{34}Cl$^-$
0.25	19	88.19	11.81
	30	100	-
0.5	22	81.07	18.93
	25	100	-
	35	100	-
0.75	24	92.56	7.44
	29	100	-
	33	100	-
1	31	100	-
2	21	86.75	13.25
	26	100	-
	32	91.98	8.02
3	23	84.79	15.21
	28	100	-
	34	73.56	26.44

4.2 [^{82}Br]-Ammonium Bromide

4.2.1 Production of [^{82}Br]-NH_4Br from NH_4Br with Natural Abundance Bromine by Thermal Neutron Activation in the UASR

[^{82}Br]-Ammoniun bromide was produced in the UASR as described in the experimental section. Four radioisotopes are formed upon irradiation of NH_4Br by thermal neutrons, *viz*, ^{80m}Br and ^{80}Br are produced from ^{79}Br and ^{82m}Br and ^{82}Br from ^{81}Br.

In the laboratory, [^{82}Br]-NH_4Br was produced from both NH_4Br with natural abundance bromine and NH_4Br with enriched ^{81}Br. Pertinent production data together with theoretical yields from 1 mg of natural abundance NH_4Br are summarized in Table 7.1. Deviations of less than 10% between the observed and calculated values were likely the result of weighing samples of very small masses.

Bromine-80 has a short physical half-life (17.68 min). Its production rate is a demonstration of this property. Saturation activity was rapidly attained after 2 hr of neutron activation. The radioactivity produced in a 3 hr production run was 34 MBq mg^{-1} of NH_4Br. The decay of the radioisotope was also rapid.

The rate of production of ^{80m}Br (T1/2 = 4.42 hr) was linear over an irradiation period of 4 hr. The maximum theoretical radioactivity produced in a 3 hr irradiation period from 1 mg of natural abundance NH_4Br was 2.8 MBq

Table 7.1: Production of radioactive bromine from natural abundance NH_4Br by thermal neutron activation in the UASR at a flux of 1 X 10^{12} n cm^{-2} sec^{-1}.

Isotope		1 hr	2 hr	3 hr	4 hr
^{80}Br					
Theor.	(MBq mg^{-1})	30	33	34	34
^{80m}Br					
Theor.	(MBq mg^{-1})	1.1	2.0	2.8	3.5
^{82}Br					
Obser.	(MBq mg^{-1})	0.16	0.31	0.45	0.62
	(MBq mM^{-1})	15.68	30.38	44.10	60.76
Theor.	(MBq mg^{-1})	0.16	0.32	0.47	0.62
^{82m}Br					
Theor.	(MBq mg^{-1})	7.3	7.3	7.3	7.3

which was estimated to decay to 1.8 MBq or 64.28% of the Amax 3 hr after EOB.

The production of ^{82}Br was a composite of those of ^{82}Br and ^{82m}Br. Saturation activity of ^{82}Br (T1/2 = 35.34 hr) was not approached under the irradiation conditions employed. The Amax for a 3 hr irradiation period was calculated to be 0.47 MBq mg^{-1} of natural abundance NH_4Br. Its decay was slow compared with the other three bromine radioisotopes reaching a level of 0.44 MBq 3 hr after EOB (93.62% of the Amax).

Bromine-82*m* with a short physical half-life of 6.05 min can be produced in abundance with short irradiation times in the UASR. Saturation activity (7.3 MBq mg^{-1}) was reached in one-half hr. Decay was very rapid because of its short

half-life.

4.2.2 Production of [^{82}Br]-NH_4Br from ^{81}Br-Enriched NH_4Br by Thermal Neutron Activation in the UASR

The experimental results of isotope production using ^{81}Br-enriched NH_4Br are tabulated in Table 7.2.

Table 7.2: Production of radioactive bromine isotopes from ^{81}Br-enriched NH_4Br by thermal neutron activation in the UASR at a flux of 1 X 10^{13} n cm^{-2} sec^{-1}.

Isotope	1 hr	2 hr	3 hr	4 hr
^{80}Br				
Theor. (MBq mg^{-1})	0.77	0.84	0.85	0.85
^{80m}Br				
Theor. (MBq mg^{-1})	0.03	0.05	0.07	0.09
^{82}Br				
Obser. (MBq mg^{-1})	0.32	0.60	0.90	1.20
(MBq mM^{-1})	31.68	59.40	89.10	118.80
Theor. (MBq mg^{-1})	0.32	0.62	0.93	1.20
^{82m}Br				
Theor. (MBq mg^{-1})	14.60	14.60	14.60	14.60

The use of enriched isotope permitted a two fold increase in the quantity of ^{82}Br produced which could be translated as a doubling of the specific activity of the labelled products.

The [^{82}Br]-NH_4Br was used without further purification. Considerable differences existed between the specific acti-

vities of radiobromide and brominated pyrimidines. With the exception of [^{82}Br]-6-BrU, the specific activities of the [^{82}Br]-brominated products were low relative to that of bromide (Table 7.3).

Table 7.3: Specific activities (MBq mM^{-1}) of [^{82}Br]-NH_4Br (3 hr thermal neutron irradiation at 1 X 10^{12} n cm^{-2} sec^{-1} and [^{82}Br]-brominated pyrimidines.

[^{82}Br]-NH_4Br	[^{82}Br]-6-BrU	[^{82}Br]-2'-BrUdR	[^{82}Br]-3'-BrUdR
44.10*	67.23†	21.53*	28.99*
89.10†			

*Natural abundance bromine
†^{81}Br-enriched NH_4Br

Irradiated bromide crystals were discolored suggesting a release of elemental bromine as a result of neutron irradiation and subsequent trapping and/or adsorption of free bromine by the crystals. The same phenomenon was also reported by Söremark[23] who suggested that an oxidative reaction by ionizing radiation and degradation of the N-Br bond by the recoil energy of activated bromine isotopes liberated free bromine which was responsible for the observed discoloration. The extent of decomposition was reported to range from 0.02 to 0.09% of the total radioactivity. Quantitation of radiolytic degradation in the SLOWPOKE reactor was not determined, but was expected to be of the

same order of magnitude.

Liberated elemental bromine reacts differently with pyrimidine bases, attacking the electron "rich" C-5 position.[138,210]

Trapped radiobromine has been suggested to recombine with decomposition products in a reaction catalyzed by heat and gamma radiation.[232] This, however, is not expected to significantly influence the radiochemical yield as only a maximum of 5% of the liberated bromine was reportedly trapped.

4.2.3 Whole-body Elimination of Radioactivity

[^{82}Br]-NH_4Br prepared from natural abundance NH_4Br was allowed to stand for 40 hr to permit decay of ^{80m}Br prior to intravenous injection into experimental animals. Whole-body radioactivity was monitored using a whole-body counter as described. Data were analysed using the *Nonlin* curve fitting program for a single component model (Fig. 4.1). Estimates of the [^{82}Br]-NH_4Br elimination parameters are listed in Table 7.4. The biological half-life of [^{82}Br]-bromide was determined to be 28.54 hr.

Söremark[231] reported a half-life of *about* 1.5 *days* for $^{82}Br^-$ in mice. The difference between the two values could be accounted for by procedural differences. Söremark's value of 1.5 days was calculated from the concentration of radiobromide in blood at different times after administration as opposed to whole-body counting in our studies. He

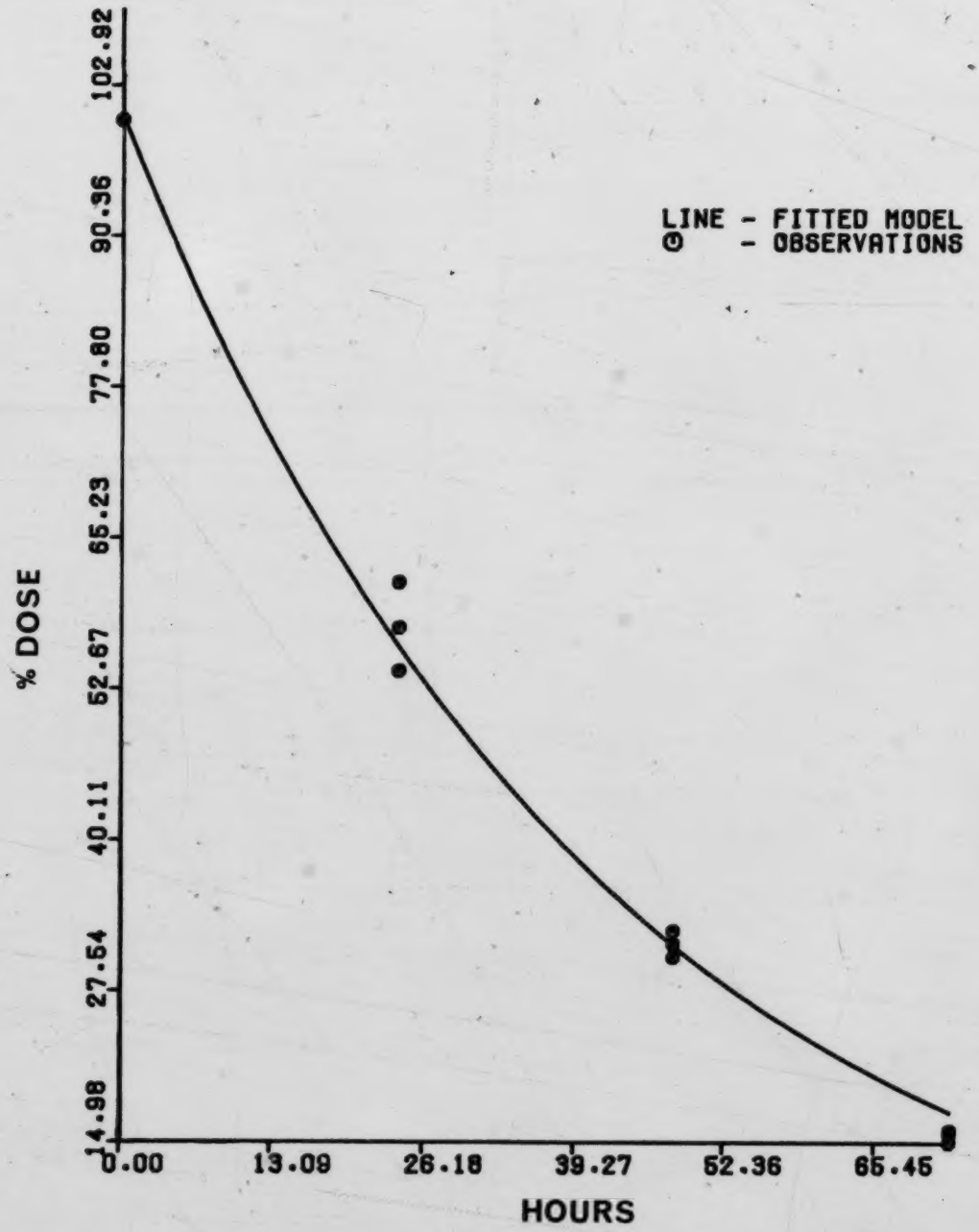


Fig. 4.1: Whole-body elimination of radioactivity by male BDF_1 mice after an iv injection of [^{82}Br]-NH_4Br. n = 3.

Table 7.4: Whole-body elimination parameters in male BDF_1 mice after an iv injection of [^{82}Br]-NH_4Br. n = 3.

	Elimination Parameters		
% Dose	100.64	±	1.26
Half-life (hr)	28.54	±	0.74

also employed 12 mice which were sacrificed after some predetermined time intervals. In our case the same 3 animals were used throughout the experimental period of 72 hr thus minimizing individual differences which were quoted by Söremark as the single most important factor in kinetics studies. A large uncertainty must accompany his value of *about* 1.5 days. An experimental duration of 128 minutes is hardly enough to determine values in the order of days. Our observed value of 28.54 ± 0.74 hr is definitely a more accurate and precise measurement. Other supportive data are provided by the biological half-lives of the long-lived components of the brominated pyrimidines (Table 7.5). Differences do exist, but are generally small except in the case of [^{82}Br]-3'-BrUdR.

Table 7.5: Biological half-lives ± S.D. (hr) of $^{82}Br^-$ and the long-lived components of [^{82}Br]-6-BrU, [^{82}Br]-2'-BrUdR and [^{82}Br]-3'-BrUdR.

$^{82}Br^-$	[^{82}Br]-6-BrU	[^{82}Br]-2'-BrUdR	[^{82}Br]-3'-BrUdR
28.54 ± 0.74	26.91 ±22.84	31.22 ±10.06	15.56 ±11.44

4.3 [^{82}Br]-6-Bromouracil (85)

4.3.1 Synthesis

In the literature non-radioactive 6-BrU was synthesized by acid hydrolysis of 2,4-dimethoxy-6-bromopyrimidine[56] and by selective debromination of 2,4,6-tribromopyrimidine.[57] Neither method is adaptable to radiochemical synthetic procedures. The hydrolytic method afforded only a 52% chemical yield because of the susceptibility of the 6-halogeno substituent to displacement by an hydroxyl group.[55,56,62,144] The chemical yield for the second method was also low (51.8%) and the radiochemical yield which is the important criterion in radiochemical synthesis is expected to be worse.

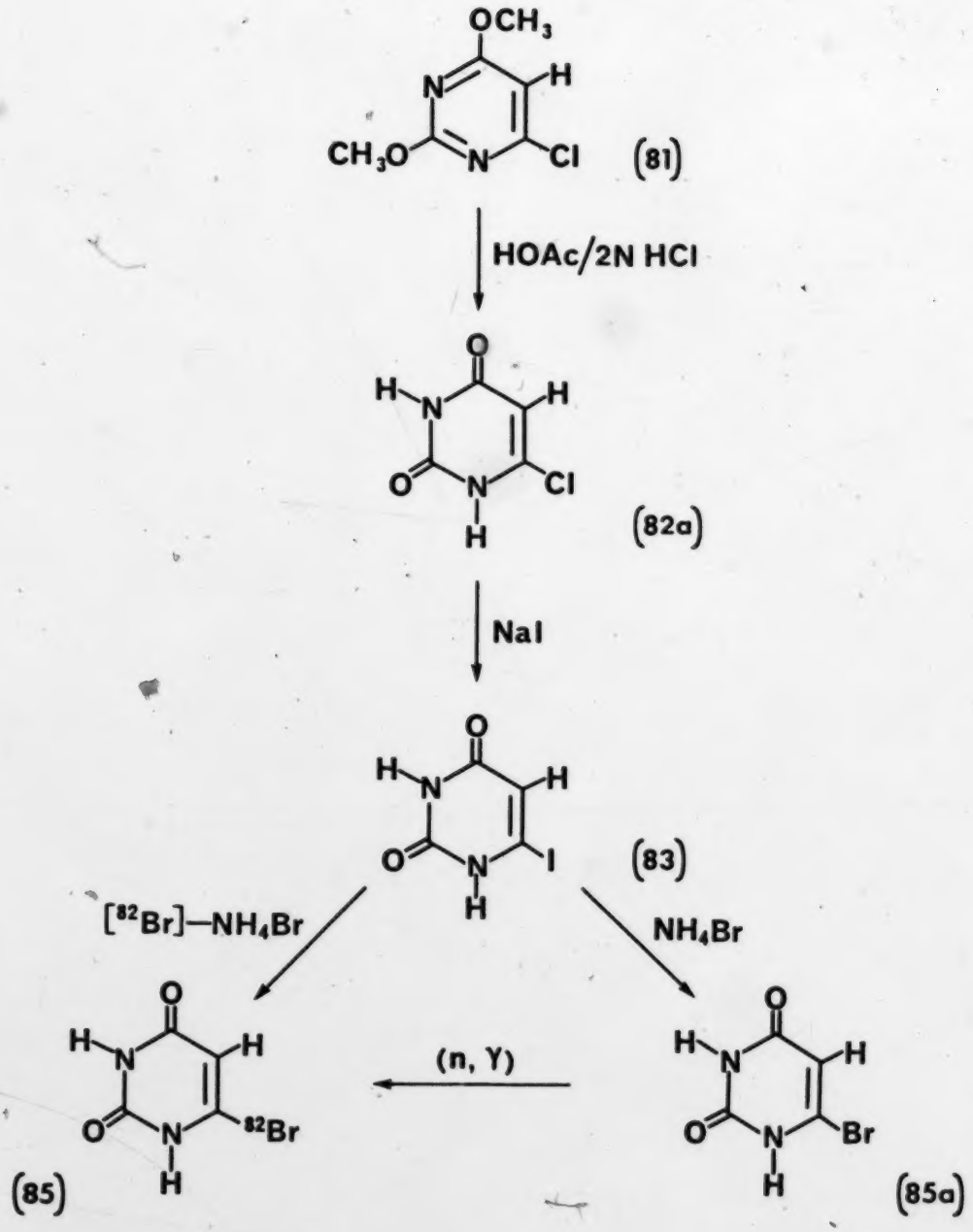
Two methods were employed for the synthesis of [^{82}Br]-6-BrU in the laboaratory:

1. Isotope exchange reaction
2. Direct neutron activation of 6-bromouracil.

The routine method of synthesis of [^{82}Br]-6-BrU in the laboratory was a bromine-for-iodine exchange reaction between [^{82}Br]-NH_4Br prepared from ^{81}Br-enriched NH_4Br and 6-IU (Scheme 6.1). Some of the reaction conditions and chemical and radiochemical yields are reported in Table 8.1. The reaction was uncomplicated and proceeded to completion with respect to 6-IU under the optimal conditions of 150°C for 1 hr. Three equivalents of bromide to one of 6-IU were generally employed.

The use of 6-ClU in the synthesis of 6-BrU *via* a bromine for chlorine exchange reaction has been explored. Results were unsatisfactory. Bromine incorporation was detected using ^{82}Br. However, separation of 6-BrU from 6-ClU was not achieved due to their similarity in TLC *Rf* value in all the development solvent systems employed.

An alternate method for the preparation of [^{82}Br]-6-BrU involved the direct neutron activation of cold 6-BrU in the UASR. Some observed and calculated yields are reported in Table 8.2. The amount of radioactivity associated with [^{82}Br]-6-BrU after 3 hr of irradiation varied from 3 to 6% of the total radioactivity produced with the majority recovered as free bromide. The irradiated sample was also observed to be discolored suggesting release of elemental bromine as a result of neutron irradiation. The covalent bond between carbon and non-radioactive bromine is a stable one. This is not the case when a radioactive bromine is involved.[3,6] The recoil energy derived from γ radiation



Scheme 6.1: Synthesis of [^{82}Br]-6-bromouracil.

Table 8.1: Synthesis of [^{82}Br]-6-BrU from 6-IU and [^{82}Br]-NH_4Br (^{81}Br-enriched NH_4Br; 3 hr irradiation at 1 X 10^{13} n cm^{-2} sec^{-1}).

[^{82}Br]-NH_4Br: 6-IU	Chemical Yield (%)	Radiochem. Yield (%)	Sp. Act. (MBq mM^{-1})
0.52	30.14	60.30	62.01
0.82	90.00	99.01	55.45
2.67	87.20	32.66	68.40
3.64	99.36	26.95	67.23

Table 8.2: Synthesis of [^{82}Br]-6-BrU by direct thermal neutron activation of natural abundance 6-BrU at a flux of 1 X 10^{13} n cm^{-2} sec^{-1}. All units are in MBq mg^{-1}.

Irradiation Time (hr)	Observed Yield	Calculated Yield	Recovery (%)
1	0.06	0.08	20
2	0.16	0.16	10
3	0.24	0.24	<10
4	0.30	0.31	<10

imparted to the radioactive bromine nuclei following capture by ^{77}Br and ^{81}Br is sufficiently energetic to rupture a carbon-bromine bond of 67 Kcal M^{-1}.[232] It is the basis of a type of Szilard-Chalmers reaction which is utilized extensively in the preparation of carrier-free bromine from inorganic and organic bromides.[214,215,233]

^{80}Br and ^{82}Br possess high energy β^- components in their decay pathways with maximum energies of 1.997 and 0.444 MeV respectively. Absorption of a fraction of energy of such magnitude could disrupt most chemical bonds.

The relative contribution of each of these processes to the observed low radiochemical yield was not determined. It is only reasonable to assume that all are negative in effect.

4.3.2 Tissue Distribution

The ability of [^{82}Br]-6-BrU to localize in different tissues was studied using male BDF$_1$ mice bearing Lewis Lung carcinomas. The mean tissue:blood ratios for the organs studied did not exceed unity except in a few instances. Fig. 5.1 represents the tissue:blood ratios of muscle, tumor and long bone. The radioactivity in the muscle was comparatively low. The maximum value was 0.16 at 6 hr. The tumor retained more of the injected radioactivity than both muscle and long bone as is evident in the higher tumor:blood ratios from time 0.08 to 12 hr. The maximum ratio attained by the tumor was 0.79 at 12 hr.

Muscle accumulated a low concentration of radioactivity. Muscle:blood ratio at 0.08 hr was 0.10 which went up to 0.16 at 6 hr. Long bone had an intermediate tissue:blood ratio value, starting at 0.12 at 0.08 hr and was 0.23 at 12 hr. The % dose g^{-1} values of blood, muscle, tumor and long bone are given in Table 8.3.

The blood at time 0.08 hr contained the highest percentage of radioactivity at 17.25% which declined to 1.71% after 12 hr. The muscle exhibited a similar tendency with 1.74% of the injected dose at 0.08 hr and 0.21% at 12 hr. More radioactivity was retained by the long bone than muscle with corresponding percentages of 2.01 and 0.49%. The radioactivity in the tumor was at a maximum of 3.29% at 1 hr and declining to 1.12% at 12 hr.

The tissue:blood ratios of GIT, stomach and skin are illustrated graphically in Fig. 5.2. The GIT showed a moderate increase in its tissue:blood ratios from 0.17 (0.08 hr) to 0.42 (12 hr). The stomach retained more radioactivity than the GIT. The initial tissue:blood ratio for stomach was similar to that of GIT at 0.13 which rose rapidly to 0.63 at 12 hr. The skin had corresponding values of 0.21 and 0.57 at 0.08 and 12 hr respectively.

The spleen was similar to the GIT in the magnitude of their tissue:blood ratios over the 12 hr period as listed in Table 8.4.

Table 8.5 is a comparison of the uptake of radioactivity per unit mass of GIT, spleen, stomach and skin. All four

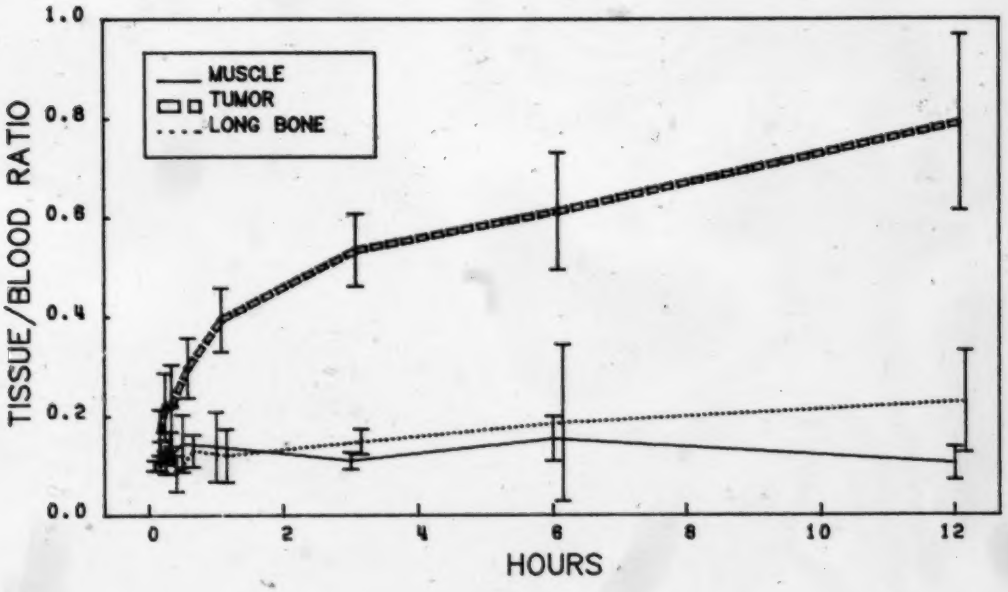


Fig. 5.1: Mean tissue:blood ratios ± 1 S.D. of muscle, tumor and long bone for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

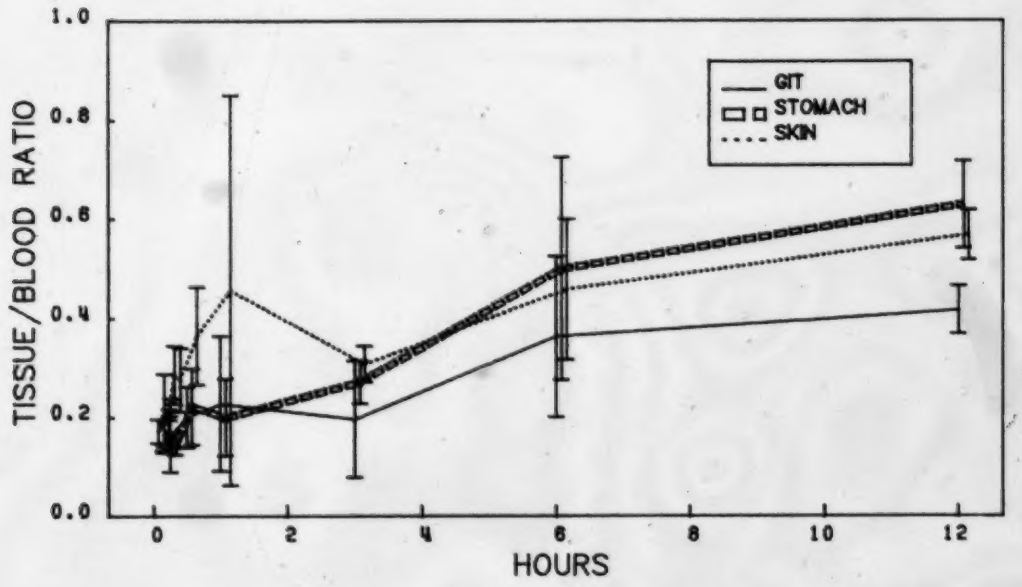


Fig. 5.2: Mean tissue:blood ratios ± 1 S.D. of GIT, stomach and skin for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

Table 8.3: Radioactivity (% dose g^{-1} tissue) in blood, muscle, tumor and long bone for male BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
Blood								
% Dose	17.25	13.02	15.76	10.60	8.35	2.42	2.28	1.71
S.D.	1.56	2.54	4.56	5.45	2.25	1.55	1.53	2.05
% S.D.	9	19	28	51	26	63	67	119
Muscle								
% Dose	1.74	1.51	1.66	1.37	1.10	0.27	0.32	0.21
S.D.	0.16	0.17	0.35	0.46	0.39	0.18	0.22	0.30
% S.D.	9	11	21	33	35	67	68	142
Tumor								
% Dose	2.84	2.75	3.23	2.96	3.29	1.26	1.30	1.12
S.D.	0.54	0.45	0.76	1.23	1.05	0.70	0.79	1.20
% S.D.	19	16	23	41	31	55	60	107
Long Bone								
% Dose	2.01	1.77	1.28	1.32	1.00	0.38	0.40	0.49
S.D.	0.22	0.19	0.45	0.61	0.42	0.31	0.38	0.63
% S.D.	10	10	35	46	41	80	94	128

oragns showed similar radioactivity retention characteristics with the exception of the skin which showed slightly higher tissue:blood ratios than the other tissues.

The tissue:blood ratios for the gall bladder, kidneys and liver are represented in Fig. 5.3. The gall bladder exhibited high ratio values of 1.10 at 0.08 hr and 0.66 at 12 hr. The corresponding ratios for the liver were 0.21 and 0.32. The kidneys were intermediate exhibiting ratios of 0.42 and 0.48 respectively. A similar tendency was observed

Table 8.4: Tissue:blood ratios ± 1 S.D. of GIT and spleen for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
GIT	0.17 ±0.02	0.22 ±0.07	0.16 ±0.08	0.20 ±0.06	0.23 ±0.14	0.20 ±0.12	0.36 ±0.16	0.42 ±0.05
Spleen	0.14 ±0.01	0.19 ±0.06	0.15 ±0.02	0.20 ±0.07	0.17 ±0.03	0.18 ±0.02	0.29 ±0.12	0.42 ±0.08

for the % dose g^{-1} as shown in Table 8.6.

The tissue:blood ratios for the lungs, heart and testicles are presented in Fig. 5.4. The lungs had the highest starting ratio (0.36) which almost doubled at 12 hr (0.67). The heart was less active than the lungs in its uptake of radioactivity with a initial ratio of 0.25 which was maintained for 12 hr except for a transient surge of radioactivity at 0.5 hr with a ratio of 0.33. At 0.08 hr the testicles had the lowest ratio of 0.05 which was increased nine folds to 0.44 after 12 hr. A similar distribution of radioactivity can also be observed for the % dose g^{-1} data shown in Table 8.7.

4.3.3 Whole-body Elimination of Radioactivity

The excretion parameters were estimated using the *Nonlin* curve fitting program for a two exponential model.

Table 8.5: Radioactivity (% dose g^{-1} tissue) in GIT, spleen, stomach and skin for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
GIT								
% Dose	2.96	2.66	2.50	1.94	1.85	0.48	0.82	0.75
S.D.	0.20	0.46	1.02	0.73	0.93	0.51	0.80	0.91
% S.D.	6	17	40	37	50	106	97	120
Spleen								
% Dose	2.42	2.39	2.33	1.90	1.42	0.46	0.60	0.70
S.D.	0.09	0.26	0.46	0.42	0.54	0.34	0.43	0.82
% S.D.	3	10	19	22	38	73	72	118
Stomach								
% Dose	2.27	2.08	2.28	2.04	1.64	0.69	0.93	0.98
S.D.	0.19	0.10	0.62	0.42	0.58	0.52	0.64	1.06
% S.D.	8	4	27	20	35	75	68	107
Skin								
% Dose	3.56	3.62	4.22	3.52	3.49	0.78	0.98	0.94
S.D.	0.49	0.37	1.00	1.28	2.31	0.58	0.75	1.06
% S.D.	13	10	23	36	66	74	76	112

Fig. 5.5 is a computer generated whole-body radioactivity elimination curve for [^{82}Br]-6-BrU. Component I and II (Table 8.8) represent the slow (long-lived) and fast (short-lived) components respectively. Component I was estimated to represent 11.81% of the injected dose with a biological half-life of 26.91 hr with corresponding values of 90.45% and 1.72 hr for component II.

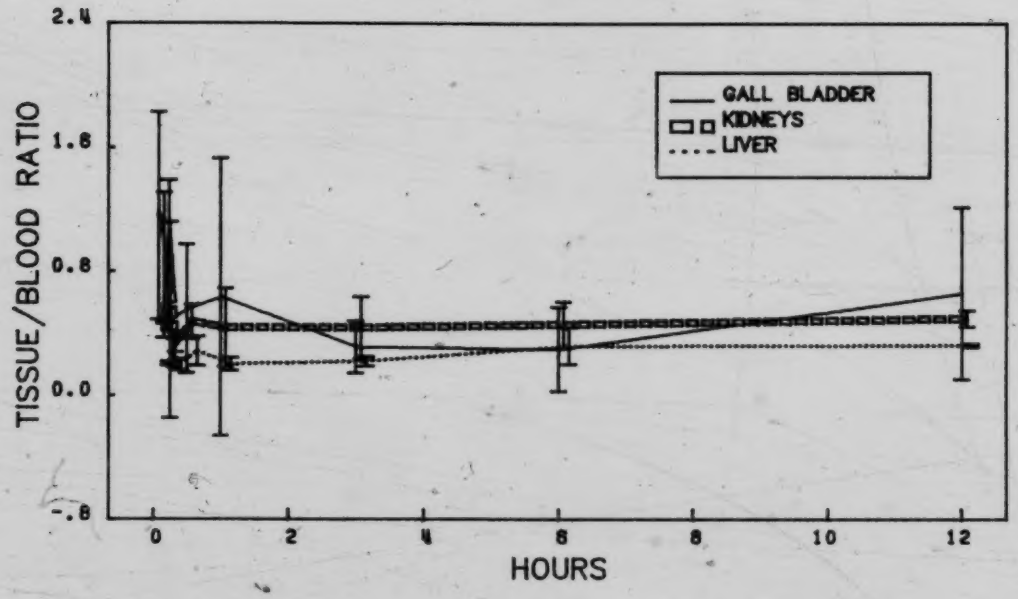


Fig. 5.3: Mean tissue:blood ratios ± 1 S.D. of gall bladder, kidneys and liver for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

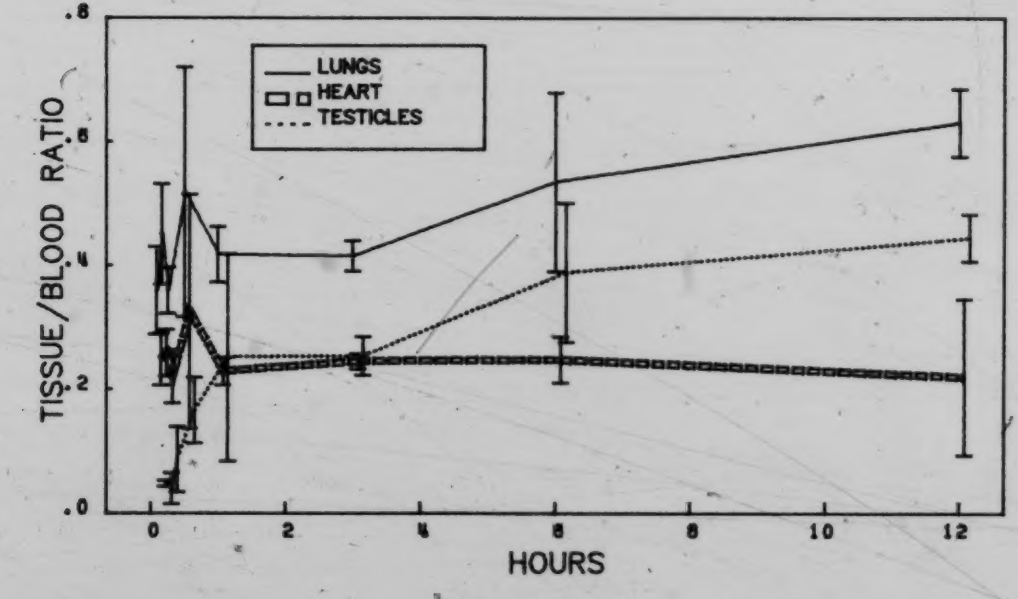


Fig. 5.4: Mean tissue:blood ratios ± 1 S.D. of lungs, heart and testicles for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

Table 8.6: Radioactivity (% dose g^{-1} tissue) in gall bladder, kidneys and liver for male BDF, mice with Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
Gall bladder								
% Dose	19.70	10.80	6.74	4.74	6.02	0.88	0.87	0.65
S.D.	10.28	3.15	7.95	2.84	9.42	0.81	1.06	0.50
% S.D.	52	29	117	59	156	91	121	76
Kidneys								
% Dose	7.24	10.88	5.23	4.66	3.80	0.96	0.95	0.86
S.D.	0.23	4.07	1.07	1.64	2.92	0.48	0.69	1.05
% S.D.	3	37	20	35	76	50	72	122
Liver								
% Dose	3.56	3.40	3.13	2.67	1.68	0.53	0.76	0.57
S.D.	0.25	0.64	1.15	0.59	0.57	0.34	0.73	0.70
% S.D.	6	18	36	22	33	62	95	122

Both [^{34}Cl]-6-ClU and [^{82}Br]-6BrU or their labels were not preferentially taken up by the tumor. The majority of the radioactivity after injection of [^{34}Cl]-6-ClU and [^{82}Br]-6-BrU was found in the kidneys and urinary bladder suggesting a major urinary route of excretion. 26% of the ^{34}Cl radioactivity in the blood was estimated to be due to $^{34}Cl^-$ (Table 6.7) and 11.81% of the ^{82}Br radioactivity was that of $^{82}Br^-$. This is a verification of the theory that instability of 6-halogenouracils is directly proportional to the electronegativity of its substituents.[49,82,83,63]

Table 8.7: Radioactivity (% dose g^{-1} tissue) in lungs, heart and testicles for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-6-BrU. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
Lungs								
% Dose	6.16	5.73	5.55	4.96	3.48	1.01	1.18	1.12
S.D.	0.99	0.52	0.96	1.79	0.99	0.64	0.87	1.37
% S.D.	16	8	17	36	28	63	74	122
Heart								
% Dose	4.29	3.30	3.40	3.03	1.90	0.58	0.55	0.49
S.D.	0.80	0.32	0.70	1.21	0.60	0.34	0.37	0.64
% S.D.	18	9	20	40	31	58	67	130
Testicles								
% Dose	0.82	0.57	1.30	1.69	2.03	0.61	0.84	0.74
S.D.	0.04	0.40	0.64	0.98	1.09	0.40	0.58	0.85
% S.D.	5	70	49	57	53	64	69	114

The formation of halides can also be demonstrated by their elimination characteristics. The blood clearance of injected [^{34}Cl]-6-ClU can be visualized as a 2 component function (Fig. 3.5 and Table 6.7) with half-lives of 1.32 and 0.31 hr representing chloride and unmetabolised [^{34}Cl]-6-ClU respectively. The serum half-life of $^{34}Cl^-$ in dog has been reported to be 2.5 days[233,234] and the daily turnover rate of Cl^- in normal adult mammal varies from 3.5 to 13%.[233] The half-life of 1.32 hr obtained in our studies was associated with very large standard deviations. It also cannot be correlated with that of a dog. Elimination of

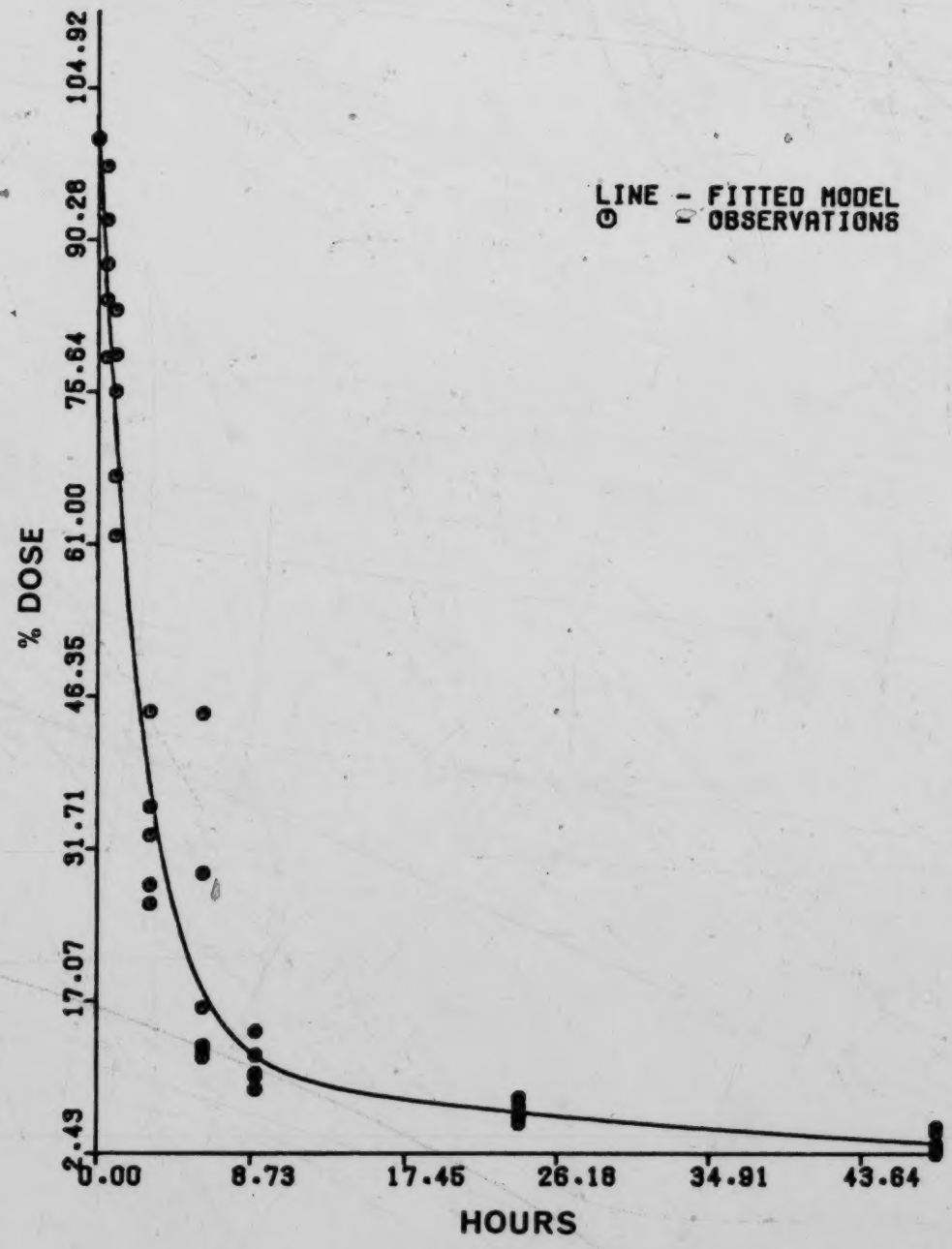


Fig. 5.5: Whole-body elimination of radioactivity from male BDF, mice after an iv injection of [^{82}Br]-6-BrU. n = 5.

Table 8.8: Whole-body elimination of radioactivity from male BDF$_1$ mice after an iv injection of [^{82}Br]-6-BrU. n = 5.

	Component I	Component II
% Dose	11.81 ± 5.70	90.45 ± 5.52
Half-life (hr)	26.91 ± 22.85	1.72 ± 0.24

exogenous compounds is a function of the physiological state of the animal. Large variations are often encountered. Our examination time of 3 hr was also inadequate for determination of values greater than 1 or 2 hr.

Chloride anion is formed as a result of dehalogenation. Its presence can be detected in body tissues with normally high chloride concentration. Chloride distribution in normal adult mammal has been reviewed by Cotlove and Hogben.[233] High chloride concentrations are found in tissues of mesenchymal origin such as erythrocytes, leukocytes, connective tissues with low metabolic rate and low requirement for organic anions. Chloride is excreted mainly by the kidneys. Chloride is also expected to accumulate in the stomach. However, the maximum observed stomach:blood ratio was 0.59 at 3 hr (Fig. 3.2). Skin with its extensive connective tissue depot is a more active tissue in terms of chloride storage. The maximum skin:blood ratio (1.32) at 3 hr was more than double that of the stomach. The presence of $^{36}Cl^-$

was also demonstrated by its tentative identification as one of the urinary metabolites of [^{34}Cl]-6-ClU and [^{34}Cl]-3'-ClUdR.

It has been suggested that bromide and chloride behave in the same manner in the secretion of HCl and HBr by gastric mucosa,[234] a view that is disputed by others.[237]

Söremark reported gastric mucosa ^{82}Br anion radioactivity of 50-80% of that of blood. Our observed value for the entire organ is 63% (12 hr) (Fig. 3.8). The two values are not directly comparable. The capacity of the stomach to concentrate [^{82}Br]-6-BrU is not known and only 11.81% of the injected dose was observed to be dehalogenated.

The biological half-life of the long-lived component (26.91 ± 22.85 hr) after an injection of [^{82}Br]-6-BrU was in reasonable agreement with that of ^{82}Br$^-$ determined earlier, suggesting *in vivo* debromination.

Many 6-substituted pyrimidines have been synthesized as potential analogues of uracil and orotic acid. Most have been found to be biologically inactive. A few are inhibitory to tumor and bacterial growth. Both [^{34}Cl]-6-ClU and [^{82}Br]-6-BrU were designed to interact with intermediary metabolic enzymes whose general requirements have been determined and reviewed in Chapter 2.

No biological data are available for 6-BrU. 6-ClU was reported to be moderately effective against *E. coli*.[33] 6-Chlorocytosine is slightly inhibitory against C1498 myelogenous leukemia *in vivo*.[43] An electron pair at the N-6

position of 6-azauridine is required to inhibit orotidylate decarboxylase.[238] An electron pair and a hydrophobic moiety at C-6 of 6-(arylalkylamino)- and 6-anilinouracils are responsible for their inhibitory action against *B. subtilis* DNA polymerase III.[43,48] Induced production of interferon has been observed in 6-phenyl substituted pyrimidines.[239] 6-Uracil sulfonamide and methylsulfones are also known to be biologically active.[54,55,240,241]

Baker *et al*[44,47,49] demonstrated that both 1-H and 3-H of uracil were complexed to the enzyme thymidine phosphorylase and that the bonding of 1-H to the enzyme was influenced by its own acidity. An electron withdrawing group at C-5 or C-6 could increase the acidity and bonding. Moreover, the effect of a substituent at C-5 appeared to be greater than that of a C-6 substituent. These positions have been suggested to be near a hydrophobic region when complexed to the enzyme, highly polar substituents could reverse this effect. The hydrophobic centers were planar and best suited for phenyl[44,48] or bicyclic or tricyclic[48] systems. Spherical halogen groups (Table 1.16) would be inadequately accommodated.

Examination of the salvage and interconversion pathways of pyrimidine nucleotides (Fig. 1.2) reveals the multiplicity of pyrimidine metabolism. Interaction with intermediary metabolism can occur at many locations. Free pyrimidine bases are coupled with furanose to form nucleosides which then form mono-, di- and triphosphates. The use of nucleo-

side is sometimes more beneficial than the corresponding free base even when the later can be metabolically transformed into the nucleoside stage. An example is 5-fluoro-2'-deoxyuridine which has a better therapeutic index than does 5-FU in the treatment of sarcoma 180,[242] Ehrlich ascites carcinoma,[242] breast lesion,[243] colon[243] and rectum tumors.[243] It was suggested that differences in activity and selectivity could be a result of differences in distribution, uptake, excretion and extent of metabolic conversion of the nucleoside as compared with the free base.[244]

Unsuccessful conversion of 6-ClU and 6-BrU to the corresponding nucleosides could be one contributing factor to the lack of biological activity of these compounds in addition to the adverse effects of an electronegative C-6 substituent as discussed above. Some evidence of the failure at the nucleoside stage is provided by analysis of urinary samples of [^{36}Cl]-6-ClU injected animals in which only the unchanged parent compound and $^{36}Cl^-$ were detected (Table 6.8). Similar analysis has not been performed with [^{82}Br]-6-BrU but whole-body elimination data (Table 8.8 and Fig. 5.5) indicate a greater stability of the bromo label than chloro (Table 6.7, Fig. 3.5) and possibly a smaller extent of metabolic conversion to the corresponding nucleoside.

4.4 1-(2'-Bromo-2'-deoxy-β-D-ribofuranosyl)uracil, [^{82}Br]-2'-BrUdR (88)

4.4.1 Synthesis

Two methods were used in the laboratory in the synthesis of [^{82}Br]-2'-BrUdR:

1. Reaction of 2,2'-anhydrouridine with ten equivalents of [^{82}Br]-NH_4Br (from natural abundance NH_4Br) afforded [^{82}Br]-2'-BrUdR. Reaction yields are reported in Table 9.1. Reaction at 100°C for 6.5 hr under anhydrous conditions appeared to be the optimum conditions for the synthesis of [^{82}Br]-2'-BrUdR. An increase in reaction temperature or reaction time did not improve the yield. The reaction did not proceed in the absence of *p*-toluenesulfonic acid. The latter was likely required for protonation of the anhydronucleoside at the N-3 position to afford a reactive protonated intermediate with enhanced electrophilicity at the C-2' position.[73]

   The 2'-bromo substituent was found to possess a *ribo* configuration[64] and the furanose ring was proposed to have an envelope conformation with C-3' *endo*.[73]

2. Direct neutron activation

   Direct activation by thermal neutrons was the routine method to introduce the ^{82}Br label in the laboratory. The results are listed in Table 9.2. The quantity of radioactivity produced was directly proportional to the length of irradiation time. The per-

Table 9.1: Synthesis of [^{82}Br]-2'-BrUdR from 2,2'-anhydro-uridine and [^{82}Br]-NH_4Br.

Temp. °C	Time (hr)	Chemical Yield (%)	Radiochem. Yield (%)	Sp. Act. (MBq mM^{-1})
100	6.5	73.4	6.31	21.53
110	5.0	50.5	4.56	12.99
120	4.0	40.0	4.06	11.10
130	1.5	24.6	2.66	5.25

Table 9.2: Synthesis of [^{82}Br]-2'-BrUdR by direct thermal neutron activation at a flux of 1×10^{13} n cm^{-2} sec^{-1}.

Irradiation Time (hr)	Activity (MBq mM^{-1})	Radiochemical Yield (%)
1	6.07	32.36
2	15.2	40
3	22.2	26.36

centages of activity recovered as [^{82}Br]-2'-BrUdR, however, were inversely proportional to irradiation time. The radiochemical yield of 26.36% after 3 hr was considerably higher than those of the other brominated pyrimidines prepared by a similar method. A comparison of the respective radiochemical yields by thermal neutron activation is given in Table 9.3. No immediate explanation is available for the large differences other than the fact that a carbohydrate moiety is not part of the structural entity of 6-BrU. However, the role of this structural dependency has yet to be explored.

Table 9.3: Radiochemical yields of brominated pyrimidines by direct thermal neutron activation (3 hr at 1 X 10^{12} n cm^{-2} sec^{-1}).

[^{82}Br]-6-BrU	[^{82}Br]-2'-BrUdR	[^{82}Br]-3'-BrUdR
<10 %	19.3 %	13.5 %

The radiochemical yields of both methods were about equivalent. Activation labelling was favored because of advantages in convenience and time saving as only purification and quality control measures were required prior to *in vivo* evaluation.

4.4.2 Tissue Distribution

[^{82}Br]-2'-BrUdR or its label was not taken up to any significant extent by the tissues investigated other than whole blood. A comparison of the tissue:blood ratios for muscle, tumor and long bone is presented in Fig. 6.1. A maximum tumor:blood ratio of 0.70 was reached 12 hr post injection. The amount of radioactivity in the muscle remained low. The maximum value of 0.23 was reached at 0.16 hr and was maintained until 0.25 hr. The level of radioactivity in the long bone did not vary appreciably throughout the 12 hr of observation with ratios betweeen 0.36 and 0.39.

The percent uptake g^{-1} of blood, muscle, tumor and long bone was compared in Table 9.4. The data supported well the tissue:blood ratio information. The highest proportion of radioactivity was found in the whole blood, followed by the tumor, long bone and muscle, in a descending order of radioactivity level. Although tumor:blood ratios were low, its percent uptake increased from 3.56% at 0.08 hr to 5.89% at 12 hr. Its significance will be discussed later.

The tissue:blood ratios for GIT, stomach and skin are presented in Fig. 6.2. The radioactivity levels in all three organs were similar with starting ratios in the range of 0.5 to 0.6 which was maintained for 12 hr. One exception was the stomach which showed a ratio of 1.03 ± 52% S.D. after 12 hr. Another organ of similar concentrating capability was the spleen which compared well with the skin (Table 9.5).

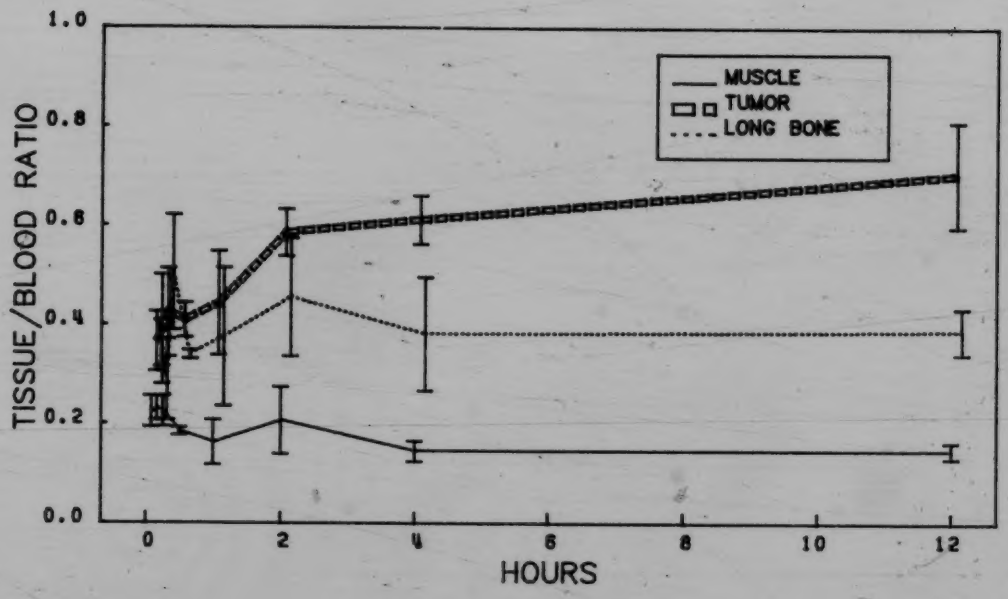


Fig. 6.1: Mean tissue:blood ratios of muscle, tumor and long bone for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

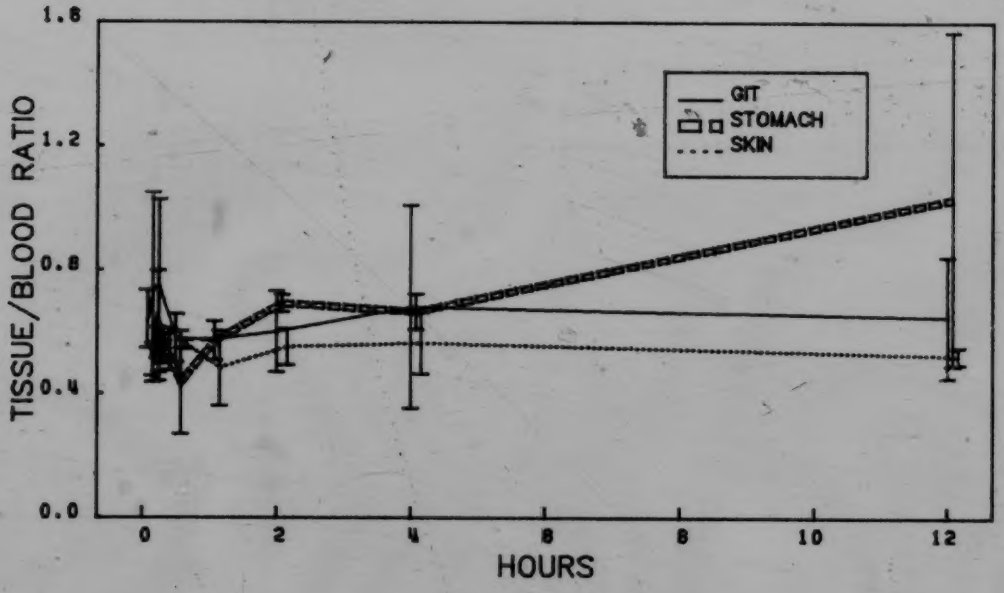


Fig. 6.2: Mean tissue:blood ratios of GIT, stomach and skin for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

Table 9.4: Radioactivity (% dose g^{-1} tissue) in blood, muscle, tumor and long bone of male BDF, mice with Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

	Time (hr)							
	0.08	0.16	0.25	0.50	1	2	4	12
Blood								
% Dose	9.65	8.24	7.03	7.36	10.37	7.66	8.31	8.26
S.D.	0.35	0.69	1.15	0.64	1.38	0.83	0.47	2.46
% S.D.	3	8	14	8	13	10	5	29
Muscle								
% Dose	2.17	1.90	1.79	1.37	1.65	1.61	1.22	1.22
S.D.	0.36	0.10	0.14	0.16	0.36	0.66	0.23	0.40
% S.D.	16	5	7	11	21	40	18	33
Tumor								
% Dose	3.56	3.25	3.48	3.01	4.52	4.51	5.10	5.89
S.D.	0.69	1.03	1.16	0.17	0.71	0.83	0.66	2.41
% S.D.	19	31	33	5	15	18	12	40
Long Bone								
% Dose	3.50	2.40	4.05	2.51	3.79	3.54	3.18	3.27
S.D.	0.42	0.73	1.13	0.27	1.18	1.25	1.00	1.35
% S.D.	12	30	27	10	31	35	31	41

Table 9.6. represents the % dose g^{-1} data for GIT, stomach, skin and spleen. The numerical values were very similar except for an uptake of 9.29% by the stomach at 12 hr.

The radioactivity in the gall bladder, kidneys and liver is presented in Fig. 6.3 and the % dose g^{-1} data are tabulated in Table 9.7. Some early biliary excretion of radioactivity was observed in the early part of the experiment after injection of [^{82}Br]-2'-BrUdR (Fig. 6.3 and Table 9.7)

Table 9.5: Tissue:blood ratios ± 1 S.D. of skin and spleen for male BDF$_1$ mice with Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	2	4	12
Skin	0.58 ±0.04	0.53 ±0.04	0.54 ±0.07	0.56 ±0.01	0.48 ±0.12	0.55 ±0.06	0.56 ±0.10	0.52 ±0.03
Spleen	0.44 ±0.07	0.53 ±0.08	0.60 ±0.14	0.48 ±0.01	0.40 ±0.08	0.52 ±0.14	0.51 ±0.10	0.50 ±0.10

representing probable elimination of either unchanged [^{82}Br]-2'-BrUdR or other labelled ^{82}Br-labelled metabolite(s) in the bile. In general large statistical variations create difficulty in interpretation of biliary data. The gall bladder:blood ratios were high from 0.08 to 1 hr with a considerable range of standard deviations. The maximum ratio of 2.02 was reached at 1 hr. Kidneys:blood ratio was 0.82 at 0.08 hr and gradually declined to 0.58 after 12 hr. The liver had the lowest radioactivity level in terms of tissue:blood ratio. The maximum ratio was 0.43 at 0.08 hr after which a ratio of about 0.3 was maintained.

The organs listed in Fig. 6.4. are lungs, heart and testicles. The ratios for lungs and heart were maintained at 0.6 and 0.4 respectively for 12 hr with little variation. A steady but slow increase in radioactivity was observed in the testicles which showed a maximum ratio of 0.60 at 12 hr.

Table 9.6: Radioactivity (% dose g^{-1} tissue) in GIT, stomach, skin and spleen for male BDF_1 mice with Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	2	4	12
GIT								
% Dose	6.19	6.05	6.11	4.23	5.91	4.64	5.70	5.67
S.D.	0.87	2.27	2.88	0.23	0.82	1.37	2.90	3.31
% S.D.	14	37	47	5	13	29	50	58
Stomach								
% Dose	4.93	5.03	4.40	3.28	5.86	5.32	5.53	9.29
S.D.	0.56	1.10	0.32	1.45	0.24	0.74	0.55	7.42
% S.D.	11	21	7	44	4	13	9	79
Skin								
% Dose	5.58	4.40	4.36	4.13	4.90	4.20	4.67	4.28
S.D.	0.38	0.43	0.80	0.46	0.73	0.16	0.71	1.06
% S.D.	6	9	18	11	14	3	15	24
Spleen								
% Dose	4.28	4.32	4.85	3.56	4.03	3.99	4.28	4.26
S.D.	0.82	0.45	1.58	0.23	0.32	1.24	0.94	2.09
% S.D.	18	10	32	6	8	31	22	49

The lungs retained twice the amount of radioactivity per unit mass as the heart relative to the blood radioactivity throughout the 12 hr of investigation (Table 9.8).

4.4.3 Whole-body Elimination of Radioactivity

Whole-body elimination of radioactivity from normal male BDF_1 mice after an iv injection of [^{82}Br]-2'-BrUdR was bi-exponential in function (Fig. 6.5, Table 9.9) as determined by the *Nonlin* curve fitting program. The two compon-

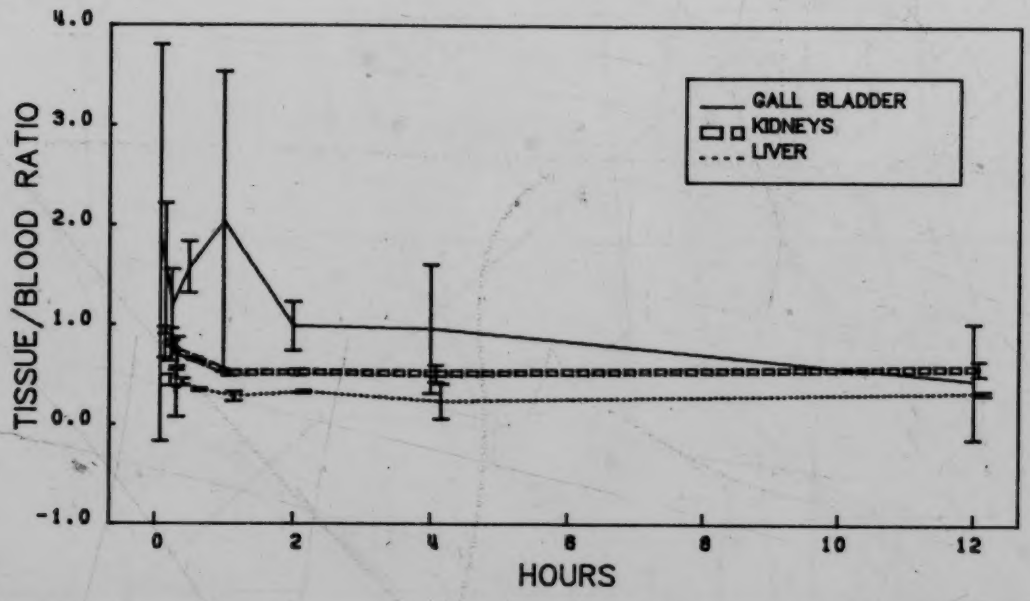


Fig. 6.3: Mean tissue:blood ratios of gall bladder, kidneys and liver for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

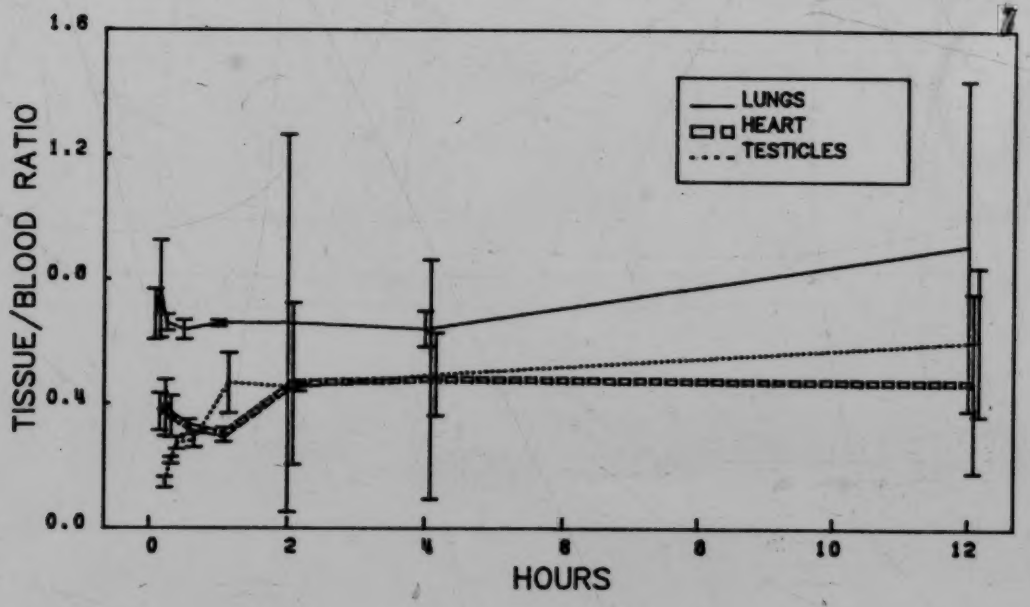


Fig. 6.4: Mean tissue:blood ratios of lungs, heart and testicles for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

Table 9.7: Radioactivity (% dose g^{-1} tissue) in gall bladder, kidneys and liver for male BDF, mice with Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	2	4	12
Gall bladder								
% Dose	17.34	12.63	9.50	11.49	22.23	7.44	8.14	2.82
S.D.	18.50	4.50	2.58	0.90	18.98	1.18	5.65	3.26
% S.D.	106	35	27	7	85	15	69	115
Kidneys								
% Dose	7.94	6.49	5.87	4.82	5.32	3.99	4.21	4.89
S.D.	1.68	0.97	1.68	0.39	0.56	0.21	0.46	2.08
% S.D.	21	14	28	8	10	5	10	42
Liver								
% Dose	4.23	2.45	3.40	2.55	2.88	2.49	1.88	2.73
S.D.	0.53	1.83	0.53	0.28	0.35	0.35	1.42	0.93
% S.D.	12	74	15	10	12	14	75	34

ents were estimated to have excretion parameters of 53.22%, 31.22 hr and 46.80%, 1.71 hr respectively.

Whole-body elimination data suggested that [^{82}Br]-2'-BrUdR was rapidly cleared by the urinary route, and at least 53.22% of the injected dose was degraded to some non-labelled products and radiobromide which could be tentatively identified by comparison with the elimination characteristics of injected [^{82}Br]-NH_4Br.

In vivo debromination could be due to cellular nucleophiles, base hydrolysis under physiological conditions or some other undetermined mechanisms.[82] A theory of *in vivo*

Table 9.8: Radioactivity (% dose g^{-1} tissue) in lungs, heart and testicles for male BDF, mice with Lewis Lung carcinomas after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	2	4	12
Lungs								
% Dose	6.64	6.25	5.29	4.68	6.82	5.13	5.32	8.27
S.D.	1.03	0.82	0.75	0.21	0.82	5.09	0.48	7.05
% S.D.	15	13	14	4	12	99	8	85
Heart								
% Dose	3.62	3.16	2.91	2.39	3.12	3.66	4.04	4.28
S.D.	0.68	0.62	0.83	0.34	0.44	2.33	3.38	3.78
% S.D.	18	19	28	14	14	63	83	88
Testicles								
% Dose	1.42	1.80	2.20	2.08	4.86	3.47	4.15	5.30
S.D.	0.12	0.10	0.39	0.22	1.31	0.44	1.28	3.62
% S.D.	8	5	17	10	27	12	30	68

metabolism of 2'-halogeno cytidines has been presented (Fig. 1.6). Similarly, [^{82}Br]-2'-BrUdR can be metabolised to Ara-U with concomitant loss of the radiolabel. Ara-U has been reported to be an active substrate of bacterial and human nucleoside phosphorylase and wheat germ phosphotransferase.[111] It also possesses anti-viral activity[248] and is toxic to mouse leukemia L5178Y cells *in vitro*.[244] The loss of radiolabel as a result of intramolecular nucleophilic displacement and the small doses employed would not permit observation of any biological activity of the test compound.

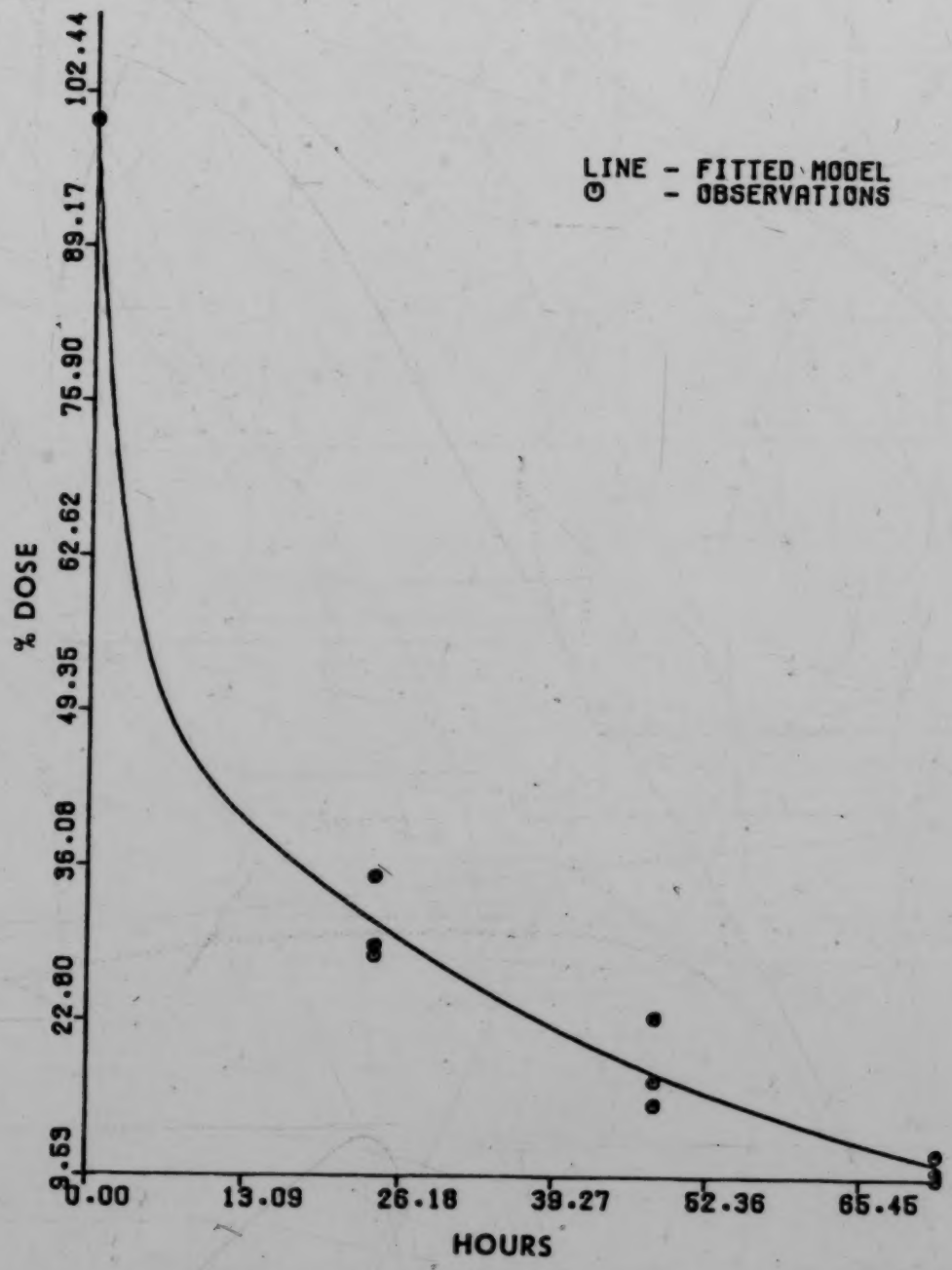


Fig. 6.5: Elimination of radioactivity from male BDF, mice after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

Table 9.9: Whole-body elimination parameters for normal male BDF_1 mice after an iv injection of [^{82}Br]-2'-BrUdR. n = 3.

	Component I	Component II
% Dose	53.22 ± 20.99	46.80 ± 21.05
Half-life (hr)	31.22 ± 10.06	1.71 ± 458.90

It has been demonstrated that a variety of 2'-substituted nucleotides of uridine and cytosine are biologically active although to a lesser extent than their corresponding unsubstituted analogues. Mn^{2+} is generally required instead of Mg^{2+} for their action and is believed to be essential for induction or maintenance of the proper conformation of the nucleotides.[105,247] The conformation of nucleotides and nucleosides is determined by the nature of their substituents, their physical sizes,[248] electronegativity[34,104,249,250] or a combination of the two properties.[73,251]

The extent of configurational changes brought about by the introduction of a bromo substituent is not known. The presence of an electronegative function at the C-2' position has been reported to pull the pucker to its side increasing the N form contribution (3'-*endo*-2'-*exo*) of the nucleoside[34] and limiting the number of possible pucker forms by imposing a potential barrier.[252] A small electronegative substituent with a small physical size such as fluorine would be

expected to dominate by its electronic interaction while a bulky but less electronegative group such as bromine could contribute significantly by its steric effects.

A change in conformation would have a greater effect on the biological activity of a nucleoside than a nucleotide. It has been suggested that the initiative nucleotide site showed a greater conformational and structural specificity than the elongation nucleotide site, and that the use of a modified nucleotide affected base-stacking.[185]

Phosphorylation of pyrimidine nucleosides to the mono-, di- or triphosphates is an often but not absolute requirement for expression of biological activities. For example 6-azauridine exerts its orotidylate decarboxylase inhibitory action only in the monophosphate form.[244] On the other hand 5'-O-sulfamoyladenosine inhibits bacterial and mammalian cell growth in its nucleoside stage.[243] Incorporation into nucleic acids occurs only at the triphosphate level.

2'-Chloro-, 2'-bromo and 2'-iodo-2'-deoxyuridine have been demonstrated to utilize human erythrocyte nucleoside transport system although at a lower rate than thymidine and 2'-fluoro-2'-deoxyuridine.[234] The rate of transport was observed to be proportional to the electronegativities of the halogens and inversely proportional to their physical sizes.

Recently Abrams *et al* reported the tissue distribution of [^{123}I]-2'-IUdR.[235] Their reported observation was in good agreement with our experimental data. Only the thyroid,

kidneys, stomach and liver accumulated a significant amount of radioactivity. 90% of the injected [^{123}I]-2'-IUdR radioactivity was excreted in 24 hr and 39% of the dose was excreted unchanged in 7 hr. Since transport across human erythrocyte membrane has been established biological inactivity of these compounds must be in part due to a suppressed or failed phosphorylation process.

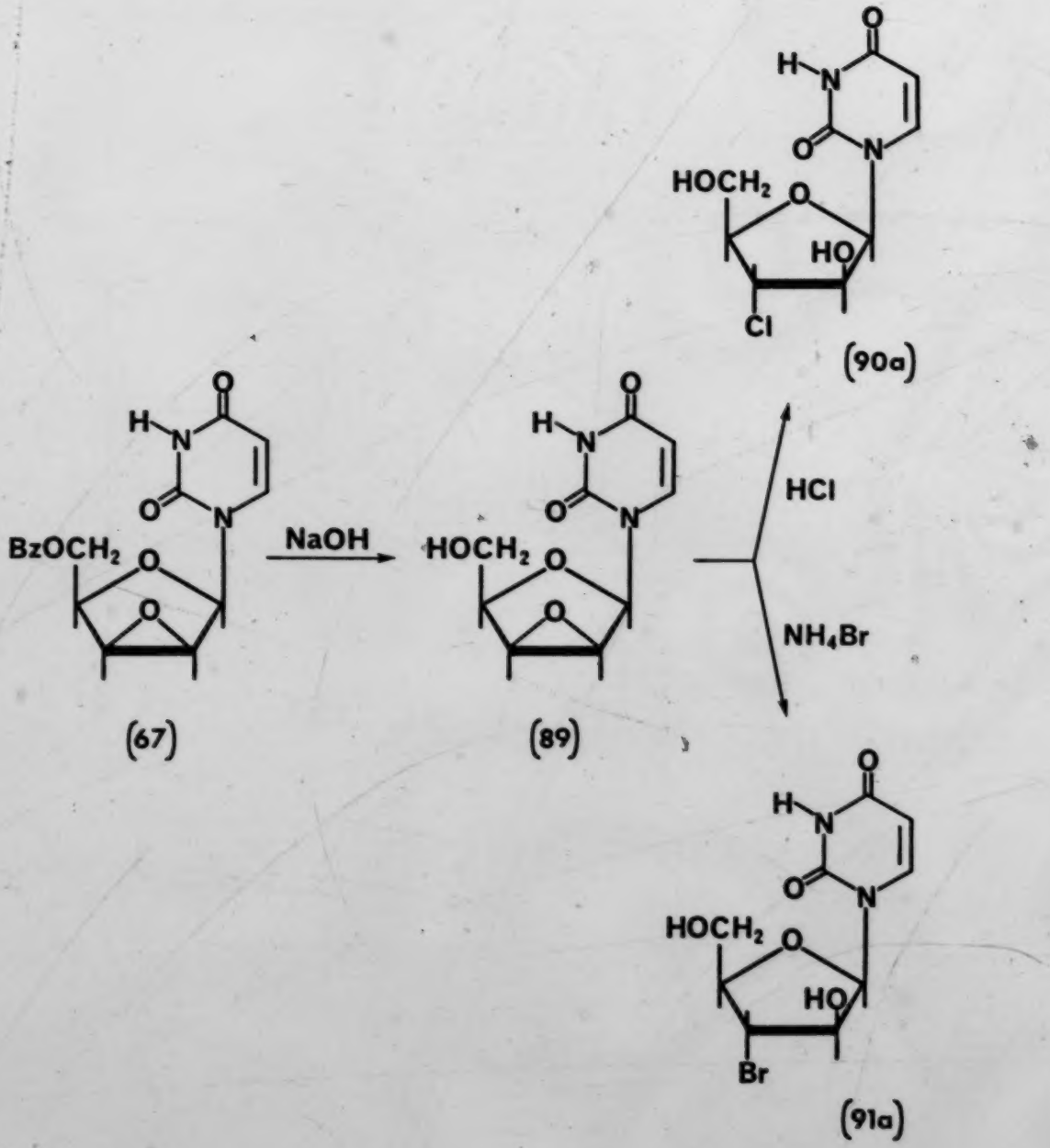
4.5 [^{36}Cl]-1-(3'-Chloro-3'-deoxy-β-D-arabinofuranosyl)-uracil, [^{36}Cl]-3'-ClUdR (90)

4.5.1 Synthesis

The synthesis of [^{36}Cl]-3'-ClUdR from the 2',3'-lyxo-epoxide of uridine 89 and the nucleophile $^{36}Cl^-$ was represented by Scheme 6.2. The optimal reaction conditions were determined to be 90°C for 1 hr using an equimolar ratio of 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (89) and [^{36}Cl]-HCl at 90°C for 1 hr (Table 10.1). The reaction was stereospecific affording mainly the 3'-deoxy-3'-substituted furanoside of the *arabino* configuration[128-132,184,186,198,223] with no detectable quantity of the 2'-isomer thus confirming reports from other laboratories.[119,120,122,188]

4.5.2 Tissue Distribution

Female BDF_1 mice were used for the tissue distribution studies of [^{36}Cl]-3'-ClUdR as male animals of the same species were not available at the time. Two criteria were



(67) (89) (90a) (91a)

Scheme 6.2: Synthesis of 1-(3'-deoxy-3'-halogeno-β-D-arabinofuranosyl)uracil.

Table 10.1: Synthesis of [^{34m}Cl]-3'-ClUdR from 2',3'-lyxo-epoxide and [^{34m}Cl]-HCl.

Temp. (°C)	Time (hr)	Chemical Yield (%)	Radiochemial Yield (%)
80	8	< 1	-
90	1	50.25	50
100	1	40.55	41

considered before the use of female animals. Transplant rejection by a different strain of mice is a distinct possibility. C57BL/6 mice are alternate hosts to the tumor, but these animals are only 50% genetically identical to BDF$_1$ mice. The benefit of sex uniformity might be outweighed by variations due to strain differences. If interesting tissue distribution patterns were observed repeat experiments would be warranted.

A significiant quantity of the injected [^{34m}Cl]-3'-ClUdR or its ^{34m}Cl label was eliminated early from experimental animals. Low levels of radioactivity contributed to the uncertainty of counting especially at 1, 2 and 3 hr. Most tissues appeared to better retain the ^{34m}Cl label than whole blood as evidenced by tissue:blood ratio value of greater than unity in many cases.

The radioactivity in muscle, tumor and long bone relative to that of whole blood is presented in Fig. 7.1. Muscle and tumor had very similar tissue:blood ratios with maximal values of 3.89 (2 hr) and 3.54 (1 hr) respectively. The radioactivity in the long bone as represented by the left tibia and left fibula was about half that of muscle and tumor, except at the 2 hr time period when the long bone:blood ratio was at a maximum of 8.70.

The quantities of radioactivity g^{-1} blood, muscle, tumor and long bone are tabulated in Table 10.2. The % dose g^{-1} was low in all cases with muscle being the most active in the uptake of radioactivity. The loss of radioactivity was rapid especially from whole blood which showed a decline of two and a half times between 0.75 and 1 hr.

The tissue:blood ratios of GIT, stomach and skin were similar and are illustrated in Fig. 7.2. The spleen was an organ that can be categorized with the above three. A comparison of the tissue:blood ratios of the spleen and skin is given in Table 10.3. The difference in tissue:blood ratios of the two organs at 1, 2 and 3 hr is likely insignificant because of the associated large standard deviations.

Fig. 7.3 is a comparison of the tissue:blood ratios of gall bladder, kidneys and liver. The gall bladder appeared to be most active in retaining radioactivity with a maximal ratio of 42.62 at 2 hr. However, the standard deviations were also large. The radioactivity in the kidneys was about

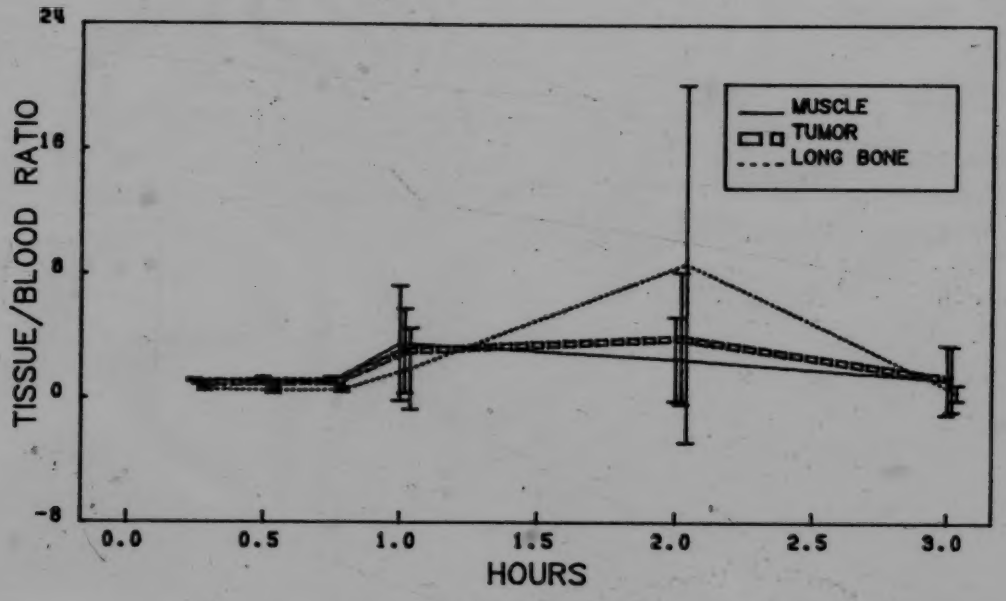


Fig. 7.1: Mean tissue:blood ratios of muscle, tumor and long bone for female BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{123}I]-3'-IUdR. n = 5.

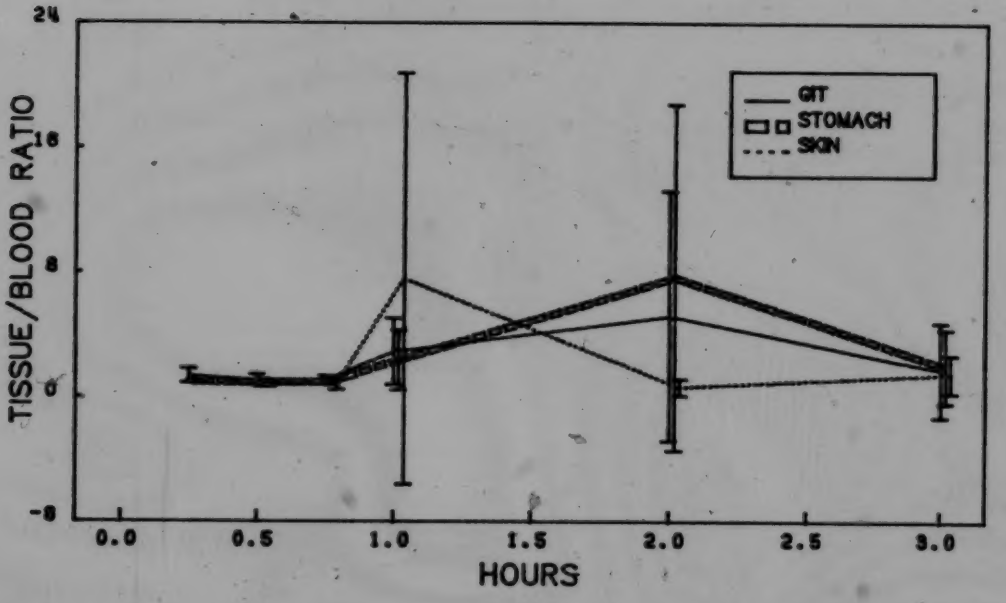


Fig. 7.2: Mean tissue:blood ratios of GIT, stomach and skin for female BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{123}I]-3'-IUdR. n = 5.

Table 10.2: Radioactivity (% dose g^{-1} tissue) in blood, muscle, tumor and long bone for female BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{131}Cl]-3'-ClUdR. n = 5.

	Time (hr)					
	0.25	0.50	0.75	1	2	3
Blood						
% Dose	0.29	0.18	0.11	0.04	0.03	0.05
S.D.	0.02	0.04	0.02	0.04	0.03	0.03
% S.D.	7	18	17	86	95	61
Muscle						
% Dose	0.32	0.20	0.12	0.06	0.02	0.01
S.D.	0.03	0.03	0.02	0.02	0.02	0.01
% S.D.	10	16	13	25	76	64
Tumor						
% Dose	0.28	0.18	0.11	0.06	0.03	0.02
S.D.	0.03	0.02	0.01	0.01	0.02	0.01
% S.D.	12	13	11	23	60	40
Long Bone						
% Dose	0.15	0.08	0.06	0.03	0.09	0.01
S.D.	0.02	0.02	0.01	0.01	0.14	0.01
% S.D.	16	28	22	45	157	68

twice that in the liver from 0.25 to 2 hr after which the ratios of both organs decreased to 0.96 and 0.98 respectively.

When tissue radioactivity (% dose g^{-1}) was compared (Table 10.5) the gall bladder was the most radioactive of the three organs. More radioactivity was found in the kidneys from 0.25 to 1 hr than in the liver, but the radioactivity levels of the two were almost identical at 2 and 3

Table 10.3: Tissue:blood ratios ± 1 S.D. in skin and spleen in female BDF_1 mice bearing Lewis Lung carcinomas after an iv injection of [^{34m}Cl]-3'-ClUdR. n = 5.

	Time (hr)					
	0.25	0.50	0.75	1	2	3
Skin	0.90 ±0.06	0.86 ±0.20	0.80 ±0.32	7.62 ±13.22	0.66 ±0.52	1.51 ±1.23
Spleen	1.06 ±0.10	0.86 ±0.16	0.88 ±0.17	2.09 ±1.83	1.64 ±1.60	0.35 ±0.17

hr.

The tissue:blood ratios of lungs and heart were presented in Fig. 7.4 and the % dose g^{-1} retention in Table 10.8. The two tissues were almost identical in all respects in terms of mean values and standard deviations.

4.5.3 Blood Clearance of Radioactivity

The radioactivity in the whole blood was analyzed using the *Nonlin* curve fitting program assuming a bi-exponential function. The results are presented in Fig. 7.5 and Table 10.7. Component I (0.03% of the injected dose g^{-1} of whole blood or 5.65% of blood radioactivity) had a half-life of greater than 1000 hr indicating incorporation of the ^{34m}Cl label or [^{34m}Cl]-3'-ClUdR. Component II or the short-lived component had a half-life of 0.27 hr and consisted of 0.5% of the injected dose g^{-1} of whole blood (94.35% of blood

Table 10.4: Radioactivity (% dose g^{-1} tissue) in GIT, stomach, skin and spleen of female BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{34}Cl]-[^{36}Cl]. n = 5.

	Time (hr)					
	0.25	0.50	0.75	1	2	3
GIT						
% Dose	0.39	0.20	0.12	0.06	0.03	0.02
S.D.	0.13	0.04	0.02	0.02	0.16	0.02
% S.D.	34	19	15	27	48	73
Stomach						
% Dose	0.28	0.16	0.11	0.05	0.06	0.05
S.D.	0.03	0.02	0.04	0.01	0.05	0.06
% S.D.	9	15	39	20	88	110
Skin						
% Dose	0.26	0.15	0.09	0.15	0.03	0.05
S.D.	0.01	0.02	0.04	0.22	0.03	0.04
% S.D.	4	15	41	144	105	80
Spleen						
% Dose	0.30	0.16	0.09	0.04	0.02	0.01
S.D.	0.03	0.03	0.01	0.00	0.01	0.01
% S.D.	10	17	9	9	58	78

radioactivity).

4.5.4 Analysis of Urinary Samples

Tentative identification of urinary metabolites was made by micro TLC in solvent system II. Twenty-five samples were analysed. [^{34}Cl]-3'-ClUdR was metabolised to several unidentified radioactive metabolites in addition to free chloride. The extent of metabolism and the number of metabolites varied considerably even within the same time

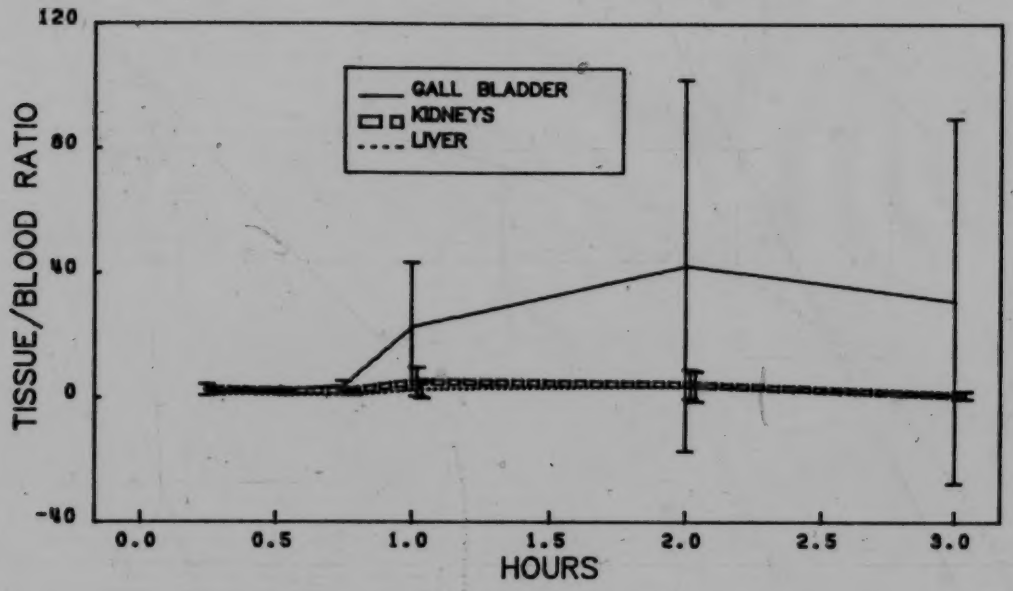


Fig. 7.3: Mean tissue:blood ratios of gall bladder, kidneys and liver for female BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{123}I]-3'-IUdR. n = 5.

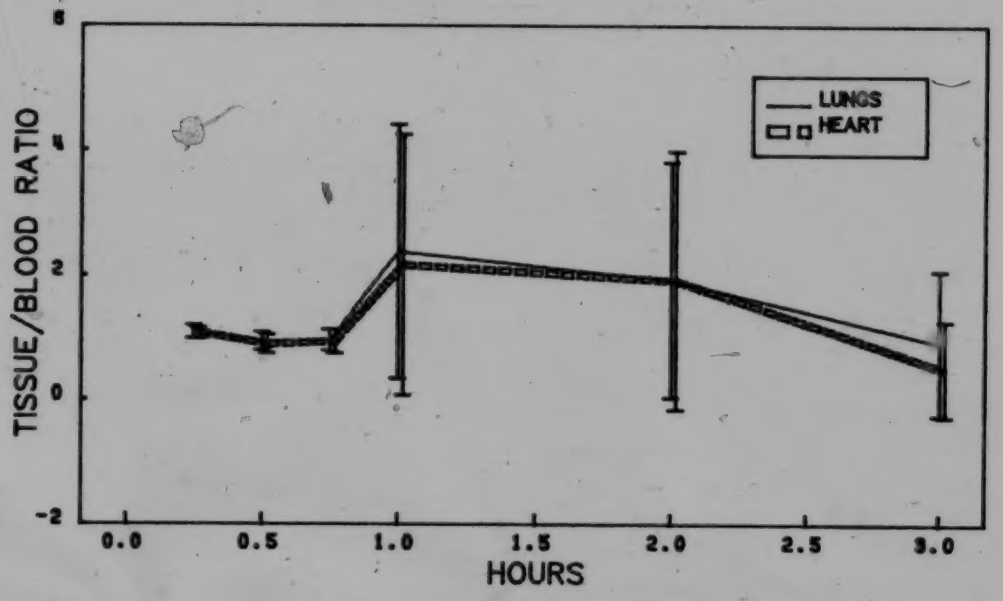


Fig. 7.4: Mean tissue:blood ratios of lungs and heart for female BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{123}I]-3'-IUdR. n = 5.

Table 10.5: Radioactivity (% dose g^{-1} tissue) in gall bladder, kidneys and liver for female BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{13}Cl]-3'-ClUdR. n = 5.

	Time (hr)					
	0.25	0.50	0.75	1	2	3
Gall bladder						
% Dose	0.71	0.52	0.43	0.47	0.26	0.28
S.D.	0.48	0.11	0.11	0.15	0.11	0.09
% S.D.	66	21	25	32	41	32
Kidneys						
% Dose	0.78	0.43	0.24	0.10	0.04	0.02
S.D.	0.07	0.12	0.04	0.01	0.02	0.01
% S.D.	8	27	19	13	66	46
Liver						
% Dose	0.34	0.18	0.10	0.05	0.03	0.02
S.D.	0.05	0.02	0.01	0.01	0.02	0.01
% S.D.	13	12	14	15	63	55

period (Table 10.8).

Examination of the tissue:blood ratio data of [^{13}Cl]-3'-ClUdR (Fig. 7.1 to 7.4 and Tables 10.2 to 10.6) revealed radioactivity levels of most tissues were at par with or greater than that of blood. If the data are divided into two sections, the radioactivity of most tissues from 15 to 45 min was of the same magnitude as that of the blood with the exception of gall bladder and kidneys which were actually more radioactive. The long bone was the lone organ with lower ratios. This suggests that tissue radioactivity was essentially that of the blood. From 1 to 3 hr all tissues

Table 10.6: Radioactivity (% dose g^{-1} tissue) in lungs and heart for female BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{36}Cl]-3'-ClUdR. n = 5.

	Time (hr)					
	0.25	0.50	0.75	1	2	3
Lungs						
% Dose	0.31	0.17	0.10	0.05	0.02	0.02
S.D.	0.03	0.02	0.01	0.01	0.01	0.01
% S.D.	9	13	9	17	50	67
Heart						
% Dose	0.30	0.16	0.10	0.04	0.02	0.01
S.D.	0.03	0.02	0.01	0.01	0.02	0.00
% S.D.	9	15	12	21	82	45

retained more radioactivity than blood. This time related phenomenon was related to the rapid elimination of injected radioactivity from the body as indicated by the blood clearance data (Fig. 7.5). 94% of blood radioactivity was computed to have a half-life of 0.27 hr (16 min).

The observed initial high radioactivity in the gall bladder and kidneys was probably due to the unmetabolised [^{36}Cl]-3'-ClUdR. Chloride is known to be excreted mainly by the kidneys[235] and only 5.65% of the blood radioactivity was estimated to be contributed by chloride. Although the extent of dechlorination was low (5.65%) the number of observed urinary metabolites was very high (Table 10.8). The labelled metabolites likely had similar elimination characteristics as the parent compound since whole-body elimination of

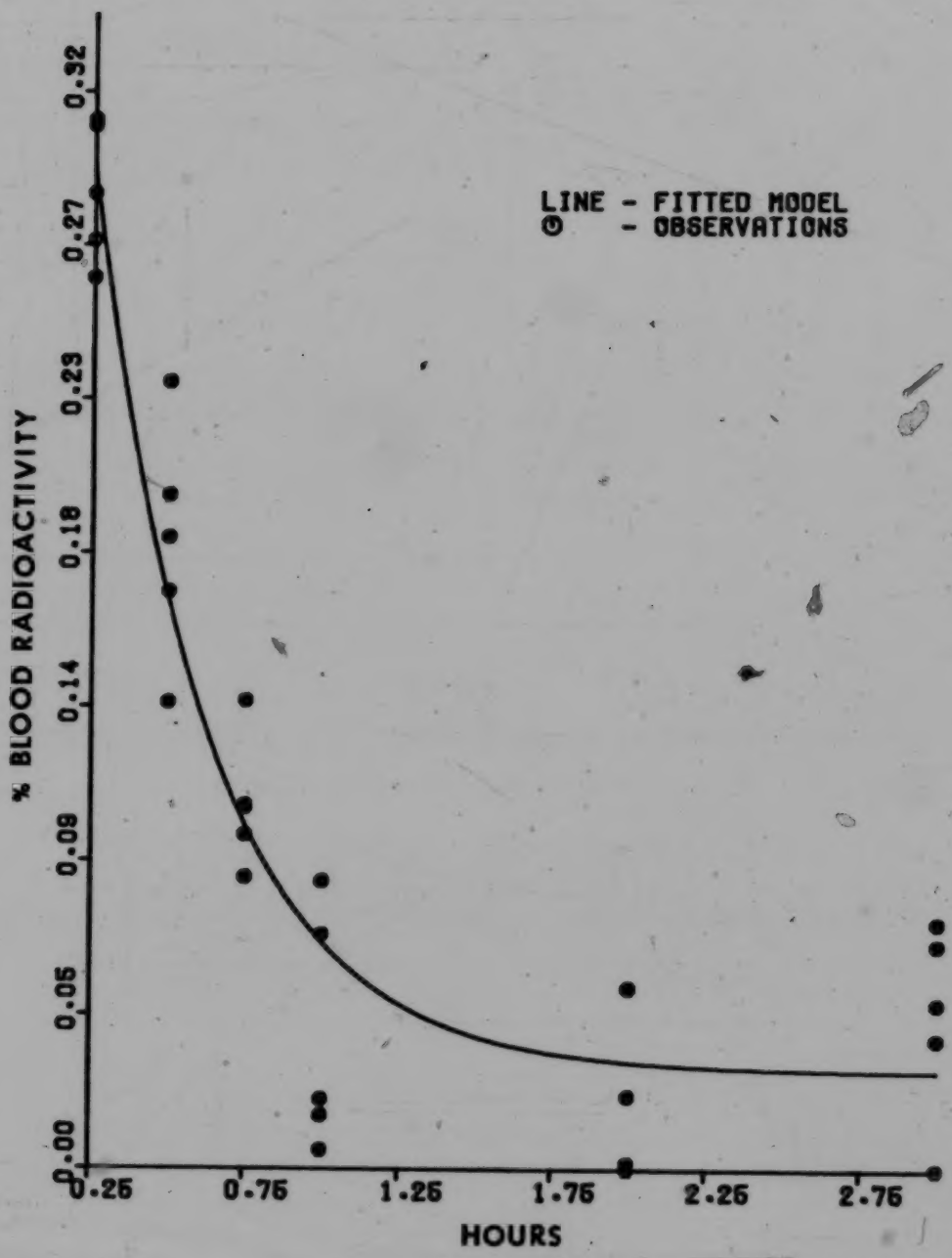


Fig. 7.5: Clearance of blood radioactivity from female BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{123}Cl]-3'-ClUdR. n = 5.

Table 10.7. Clearance of blood radioactivity in female BDF$_1$ mice with Lewis Lung carcinomas after an iv injection of [^{34}Cl]-3'-ClUdR. n = 5.

	Component I	Component II
% Dose	0.03 ± 0.05	0.50 ± 0.05
Half-life (hr)	> 1000	0.27 ± 0.08

radioactivity was best described by a bi-exponential model (Fig. 7.5).

4.6 [^{82}Br]-1-(3'-Bromo-3'-deoxy-β-D-arabinofuranosyl)-uracil, [^{82}Br]-3'-BrUdR (91)

4.6.1 Synthesis

The title compound was prepared by two 2 methods:

1. Reaction of 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (89) with [^{82}Br]-NH_4Br

   Reaction of the 2',3'-epoxide 89 with 2 equivalents of [^{82}Br]-NH_4Br which provided the acidic medium necessary for catalysis of the nucleophilic reaction afforded [^{82}Br]-3'-BrUdR. Results of the reaction are listed in Table 11.1. The reaction conditions of 150°C for 1 hr were judged to be optimal because of satisfactory chemical yield. Actual chemical yields sometimes varied as the release of solvent during reaction was

Table 10.8: Urinary metabolites of [^{36}Cl]-3'-ClUdR expressed as percentages of urinary radioactivity.

Time (hr)	Animal	[^{36}Cl]-3'-cludr	^{36}Cl$^-$	Unidentified
0.25	7	66.41	33.59	-
	13	32.21	8.52	32.76; 26.50
	22	93.65	-	6.35
0.50	1	75.28	24.7	-
	19	60.75	14.80	24.45
	21	88.16	-	11.84
	29	77.96	-	22.04
0.75	5	26.44	73.56	-
	8	86.98	8.42	4.59
	14	28.19	8.86	10.62; 52.33
	23	88.03	-	11.97
	28	62.69	34.71	2.60
1	2	24.06	75.94	-
	9	5.16	7.70	27.83; 59.31
	15	29.86	12.40	23.94; 33.80
	16	19.39	7.17	73.43
	20	64.82	6.53	16.53
2	4	21.57	45.69	32.74
	10	-	21.81	78.19
	24	48.20	28.31	23.49
	25	56.64	28.44	14.91
3	6	-	88.12	2.40; 9.48
	17	3.49	-	10.05; 15.41; 18.10; 52.94
	18	66.49	10.33	10.71; 12.46
	26	29.16	50.53	20.31

difficult to control. As in the case of [^{34}Cl]-3'-ClUdR only the 3'-isomer was detected during work-up of the reaction mixture, again confirming the stereospecificity of the reaction.

2. Direct thermal neutron activation of 3'-BrUdR in the UASR

Attempts to produce [^{82}Br]-3'-BrUdR of high specific activity from 3'-BrUdR by direct thermal neutron activation were unsuccessful (Table 11.2). The radiochemical yield was low as in the case of 6-BrU and was probably for the same reasons. Most of the radioactivity produced was found to be associated with free bromide which could be conceivably formed by radiolysis of the irradiated product.

4.6.2 Tissue Distribution

[^{82}Br]-3'-BrUdR and its label were evenly distributed throughout most of the organs of the experimental animals. Kidneys and gall bladder were the only organs with high concentrations of radioactivity. Low levels of radioactivity were detected in muscle, tumor and long bone (Fig. 8.1) with radioactivity in the tumor approaching that of whole blood (maximum ratio of 1.01 at 0.25 hr). Tumor also possessed more radioactivity towards the end of the observation period (0.66 at 12 hr) than the others. The maximum ratios exhibited by muscle and long bone were 0.98 (0.25 hr) and 0.54 (0.5 hr) respectively.

Table 11.1: Synthesis of [^{82}Br]-3'-BrUdR from 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil and [^{82}Br]-NH_4Br.

Temp. (°C)	Time (h)	Ratio*	Chemical Yield(%)	Radiochem. Yield (%)	Sp. Act. (MBq mM^{-1})
120	0.5	1.20	40.00	33.33	28.66
120	1	1.50	50.55	33.70	30.15
120	2	2.29	69.07	30.16	27.65
150	0.5	2.10	51.33	24.44	29.35
150	1	2.00	67.14	33.57	28.99
150	1.5	2.25	72.07	32.03	25.33

*Ratio of NH_4Br:epoxide

Table 11.2: Synthesis of [^{82}Br]-3'-BrUdR by direct thermal neutron activation in the UASR at a flux of 1 X 10^{12} n cm^{-2} sec^{-1}

Activation Time (h)	Radiochemical Yield (%)	Sp. Act. (MBq mM^{-1})
1.25	12.92 ± 1.08	2.88 ± 0.78
2	12.96 ± 0.87	4.78 ± 0.83
3	13.50 ± 1.37	8.10 ± 0.95

A comparison of the % dose g^{-1} data revealed a very high radioactivity in whole blood from which clearance was also rapid with elimination of greater than 50% in 0.25 hr (Table 11.3). The tumor was more effective in retaining its radioactivity with a final ratio of 0.66. Long bone was intermediate in terms of % dose g^{-1}.

The tissue:blood ratios for GIT, stomach and skin are given in Fig. 8.2. with maximum ratios of 1.11 (0.25 hr), 0.91 (1 hr) and 1.48 (0.5 hr) respectively. A two-fold decrease in the tissue:blood ratios for the GIT was observed over a 12 hr period while those of stomach and skin remained relatively stable. The tissue:blood ratios for the GIT and spleen were similar. Their respective tissue:blood ratios are compared in Table 11.4.

The percentage of the dose retained g^{-1} GIT, stomach, skin and spleen are compared in Table 11.5. The amount of radioactivity was lowest in skin. Radioactivity levels in the GIT, stomach and spleen were of a similar order of magnitude.

The tissue:blood ratios of gall bladder, kidneys and liver are presented in Fig. 8.3. The gall bladder contained the most radioactivity relative to whole blood with a maximum of 8.66 at 1 hr. Radioactivity also remained high to the end of the observation period (1.05 at 12 hr). The highest ratio observed for kidneys was 3.98 at 0.08 hr. Liver had the lowest radioactivity content and was at a similar level as whole blood up to 0.25 hr after which the

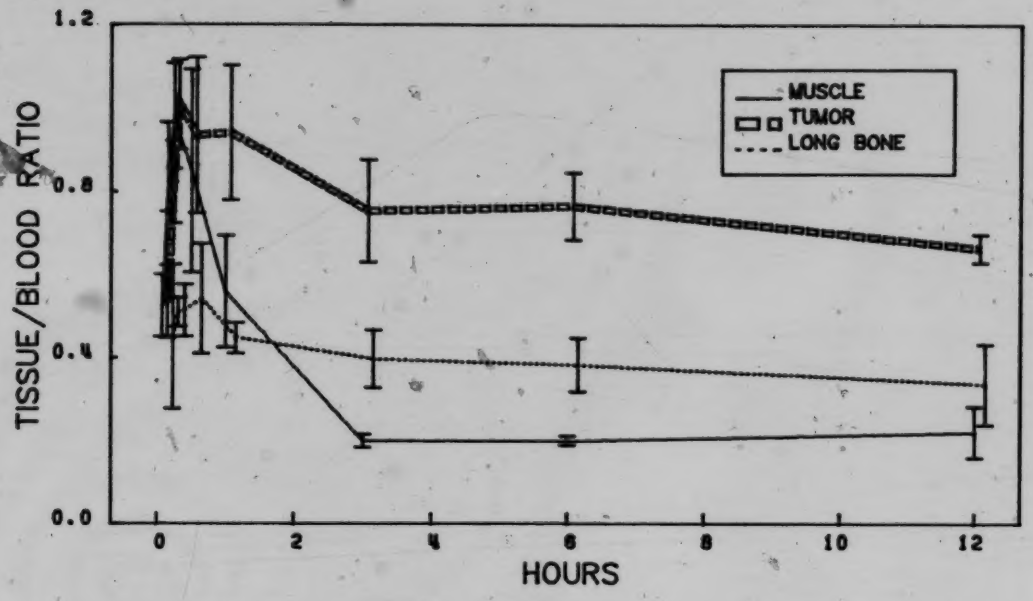


Fig. 8.1: Mean tissue:blood ratios of muscle, tumor and long bone for male BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

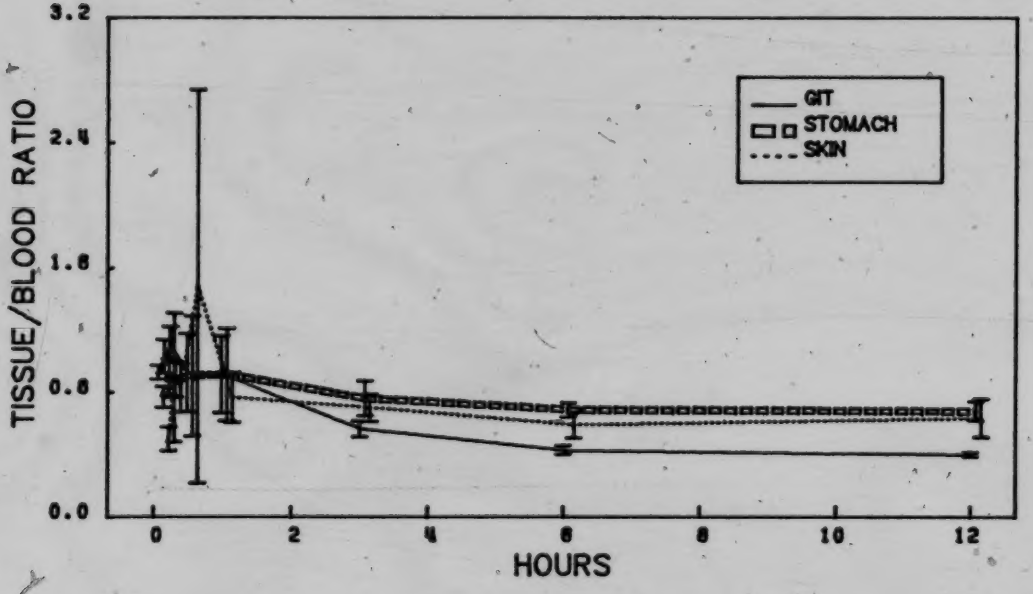


Fig. 8.2: Mean tissue:blood ratios of GIT, stomach and skin for male BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

Table 11.3: Radioactivity (% dose g^{-1} tissue) in blood, muscle, tumor and long bone for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
Blood								
% Dose	5.82	3.50	2.33	2.31	1.35	0.93	0.93	0.65
S.D.	1.23	0.93	0.50	1.06	0.63	0.49	0.24	0.10
% S.D.	21	26	21	45	47	52	26	14
Muscle								
% Dose	3.02	2.94	2.24	1.76	0.71	0.19	0.18	0.14
S.D.	0.61	0.54	0.31	0.39	0.26	0.10	0.05	0.04
% S.D.	20	18	13	22	36	55	26	24
Tumor								
% Dose	3.07	2.89	2.32	2.01	1.19	0.68	0.70	0.43
S.D.	0.58	0.87	0.35	0.58	0.39	0.34	0.16	0.06
% S.D.	18	29	15	28	32	49	23	14
Long Bone								
% Dose	2.56	1.78	1.17	1.23	0.59	0.36	0.36	0.22
S.D.	0.77	0.47	0.15	0.60	0.24	0.18	0.14	0.06
% S.D.	30	26	12	48	40	50	39	28

ratios declined rapidly.

In Table 11.6 percentages uptake of radioactivity g^{-1} of gall bladder, kidneys and liver are compared. Twenty-three percent of the injected dose was detected in the kidneys at 0.08 hr and declined sharply to 0.34% after 12 hr. Gall bladder contained more radioactivity g^{-1} tissue than liver. The significance of this oservation has been discussed earlier.

Table 11.4: Tissue:blood ratios of GIT and spleen for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
GIT								
Ratio	0.93	1.03	1.11	0.92	0.92	0.57	0.43	0.40
S.D.	0.04	0.11	0.11	0.25	0.25	0.05	0.03	0.02
Spleen								
Ratio	0.81	0.99	1.00	0.76	0.58	0.46	0.39	0.44
S.D.	0.06	0.08	0.12	0.13	0.06	0.05	0.02	0.04

Fig. 8.4 represents the tissue:blood ratios for lungs, heart and testicles. The ratios of lungs and heart were similar with maxima of 1.00 and 1.05 respectively at 0.08 hr and decreased with time. In contrast to the lungs and heart the radioactivity in the testicles increased with time. The ratio was 0.16 at 0.08 hr and was 0.53 after 12 hr with a maximum of 1.28 at 1 hr (Fig. 8.4). The lungs and heart contained relatively large quantities of radioactivity at 0.08 hr with percentages of 5.82 and 6.08 respectively (Table 11.7). Corresponding values after 12 hr were 0.43 and 0.20. The lungs usually retained more radioactivity than the heart due to a more extensive vasculature and lower tissue density of the lungs. The nature of an exceptional case with the 3'-halogeno arabinosides is not known.

Table 11.5: Radioactivity (% dose g^{-1} tissue) in GIT, spleen, stomach and skin for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
GIT								
% Dose	5.36	3.54	2.54	1.93	1.11	0.51	0.40	0.26
S.D.	0.98	0.64	0.33	0.42	0.27	0.22	0.10	0.04
% S.D.	18	18	12	21	24	42	24	13
Spleen								
% Dose	4.71	3.41	2.30	1.65	0.76	0.42	0.36	0.29
S.D	0.92	0.65	0.49	0.52	0.32	0.19	0.10	0.05
% S.D.	19	18	21	31	41	45	27	18
Stomach								
% Dose	4.43	3.05	2.02	1.86	1.14	0.71	0.64	0.44
S.D.	0.75	0.48	0.33	0.50	0.40	0.40	0.18	0.09
% S.D.	16	15	16	26	35	56	27	19
Skin								
% Dose	2.86	3.00	1.87	2.90	0.96	0.62	0.54	0.41
S.D.	0.43	1.18	0.20	1.99	0.31	0.22	0.14	0.05
% S.D.	15	39	10	68	32	35	25	12

The percentage of the injected dose in the testicles also declined from 0.90 at 0.08 hr to 0.39 after 12 hr. However, the rate of decline was much slower than that for lungs, heart and blood.

4.6.3 Whole-body Elimination of Radioactivity

Analysis of whole-body radioactivity was performed using the *Nonlin* program assuming a bi-exponential excretory function. [^{82}Br]-3'-BrUdR exhibited a high rate of urinary

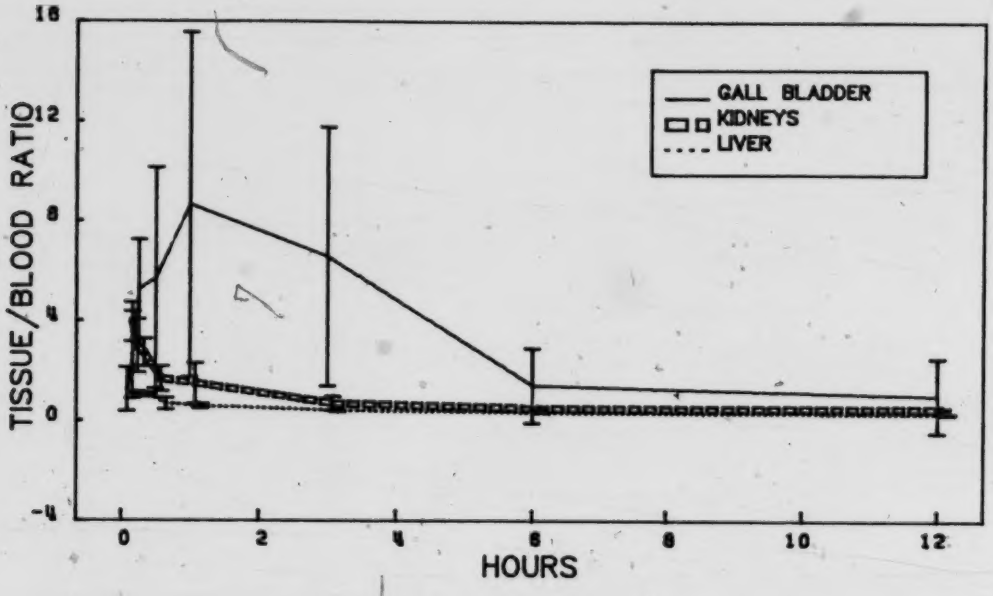


Fig. 8.3: Mean tissue:blood ratios of gall bladder, kidneys and liver for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

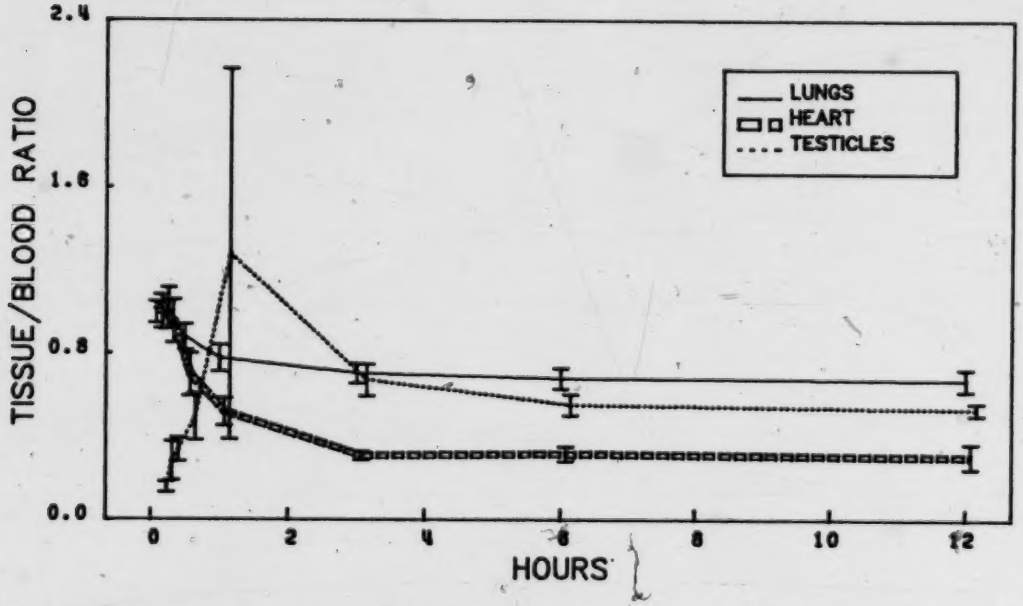


Fig. 8.4: Mean tissue:blood ratios of lungs, heart and testicles for male BDF, mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

Table 11.6: Radioactivity (% dose g^{-1} tissue) in gall bladder, kidneys and liver for male BDF_1 mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
Gall Bladder								
% Dose	7.16	8.43	11.60	10.02	9.46	4.79	1.30	0.61
S.D.	5.20	3.35	2.74	4.81	5.58	3.45	1.22	0.76
% S.D.	72	39	23	48	59	72	94	123
Kidneys								
% Dose	22.77	10.21	6.24	3.48	1.72	0.61	0.49	0.34
S.D.	5.30	3.44	1.29	0.69	0.42	0.21	0.11	0.05
% S.D.	23	33	20	19	24	34	21	14
Liver								
% Dose	6.69	3.67	2.36	1.46	0.77	0.39	0.33	0.20
S.D	1.53	0.79	0.50	0.60	0.28	0.17	0.10	0.03
% S.D.	22	21	21	40	35	42	31	13

excretion. The fitted curve is shown in Fig. 8.5. The estimated excretion parameters of the 2 components are listed in Table 11.8. A long-lived and short-lived component were identified with percentages of 10.73 and 91.10 and half-lives of 15.56 and 0.25 hr respectively. The extent of dehalogenation was also small with 8.9% estimated debromination (half-life 15.6 hr).

The biological activity of 3'-ClUdR has not been reported. Its corresponding bromo, iodo, azido and thiocyano analogues of uridine and cytidine were shown to be ineffective against L5178Y cells *in vivo*. The lack of activity was

Table 11.7: Radioactivity (% dose g^{-1} tissue) in lungs, heart and testicles for male BDF$_1$ mice bearing Lewis Lung carcinomas after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

	Time (hr)							
	0.08	0.16	0.25	0.5	1	3	6	12
Lungs								
% Dose	5.82	3.40	2.35	1.99	1.02	0.65	0.63	0.43
S.D.	1.14	0.73	0.43	0.79	0.42	0.32	0.16	0.04
% S.D.	19	21	18	39	40	49	24	8
Heart								
% Dose	6.08	3.40	2.23	1.53	0.68	0.29	0.29	0.20
S.D	1.21	0.71	0.59	0.48	0.28	0.16	0.06	0.03
% S.D.	19	20	26	31	41	55	19	14
Testicles								
% Dose	0.90	0.98	0.76	1.06	1.68	0.60	0.51	0.35
S.D.	0.19	0.33	0.07	0.29	1.47	0.23	0.14	0.05
% S.D.	20	33	9	27	87	38	27	13

believed to be due to the absence of an *arabino* 3'-OH group[1,3] which was reported to be necessary for binding to thymidine phosporylase.[1,33]

Many of the arguments for 2'-substituted nucleosides in terms of steric and electronic effects can also be applied to the 3'-position.[34,281]

The configuration of 3'-fluoro and 3'-chlorouridine was reported to have a twist conformation with the ring oxygen *endo* and C-1' *exo* which is significantly different from unsubstituted ribonucleoside and deoxyribonucleoside (envelope with C-2' *endo*).[3] As a result of conformational

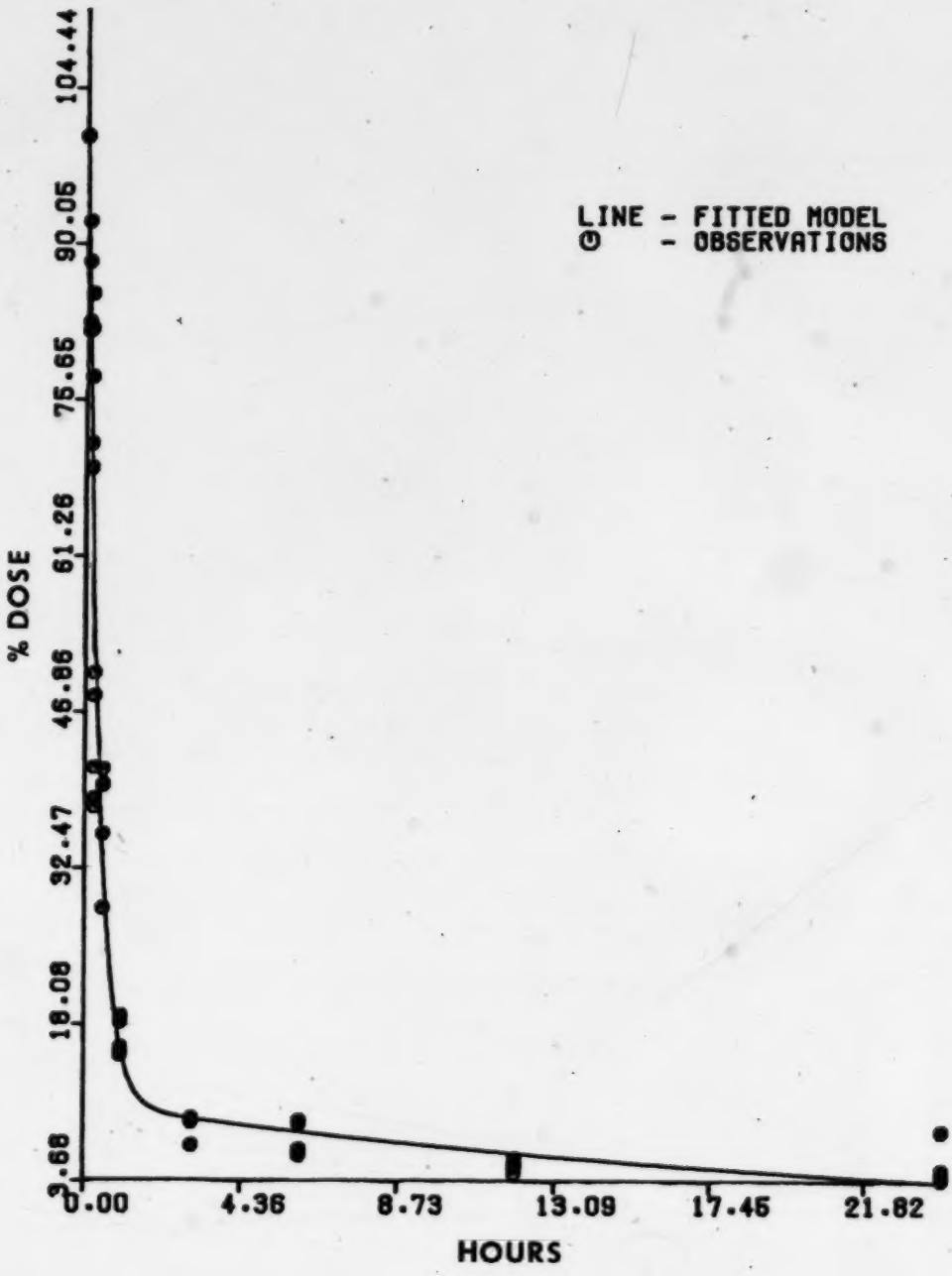


Fig. 8.5: Elimination of radioactivity from male BDF, mice mice after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

Table 11.8: Whole-body elimination parameters of male BDF$_1$ mice after an iv injection of [^{82}Br]-3'-BrUdR. n = 5.

	Component I	Component II
% Dose	10.73 ± 2.93	91.10 ± 3.44
Half-life (hr)	15.56 ± 11.44	0.25 ± 0.02

changes substrate-enzyme complex formation might be unfavorably affected. Phosphorylation could be inhibited and uptake not observed.

However, in the cytidine series, the 3'-fluoro and 3'-bromo *xylo* derivatives were reported to be active against L1210 leukemia cells *in vitro*.[113]

Watanabe *et al*[256] also reported that 3'-chloro-, 3'-bromo- and 3'-iodoxylosyl nucleosides of cytosine and 5-fluorocytosine were active against L5178Y and P815 mouse leukemia cells *in vitro*.[256] The action was described as "double-barreled masked precursors" in that the said compounds behaved similarly to Ara-C in Ara-C sensitive cell lines and acted as 5-fluoropyrimidines in Ara-C resistant lines. The C-3' position was hypothesized to undergo an intramolecular nucleophilic attack by the 2'-OH to form a 2',3'-xyloepoxide which was readily converted to the corresponding 2,2'-anhydronucleosides by the nucleophilic action of the O^2 atom. Further metabolism afforded Ara-C or Ara-FC

which were responsible for the observed biological activity.[256] 2',3'-Xyloepoxide and 2,2'-anhydrofuranoside have also been proposed to be the intermediates in the synthesis of 1-β-D-arabinofuranosyl-5-fluorouracil.[111] It was also suggested that cytotoxicity of the 3'-halogenoxylosyl nucleosides is dependent upon the "leaving" capacity of the halogeno group.[256] The 3'-fluoro analogues were hardly cytotoxic because of a strong C-F bond. The 3'-chloro analogues were less active than the corresponding 3'-bromo analogues. The 3'-iodo analogues were slightly less toxic than the 3'-bromo analogues because of steric effects.[256]

If the "double-barreled masked precursors" concept were applicable to the 3'-halogeno arabino nucleosides no observable biological activity for [^{36}Cl]-3'-ClUdR and [^{82}Br]-3'-BrUdR could be expected. The formation of the 2',3'-epoxide is necessarily preceded by dehalogenation which would render impossible observation of any tumor localization activity. The formation of 2,2'-anhydronucleoside and Ara-U is unlikely as the 2',3'-lyxoepoxide has been known to be stable toward intramolecular nucleophilic displacememt by the O^2 atom.

Most research on halogenated pyrimidines has been centered on their anti-viral, anti-bacterial, anti-fungal, anti-tumor, enzyme-inhibiting and interferon-inducing activities. The potential of these compounds in diagnostic oncology is virtually unexplored until now. Ideal therapeutic agents are both target specific and cytotoxic. Diagnostic

radiopharmaceuticals differ in that target specificity is the main deciding factor because of the subpharmacological doses employed. The compounds tested in the present project do not appear to demonstrate fufillment of this quality. The tumor:blood ratios were low and in most instances below unity.

Recent development in image reconstruction technique has made possible employment of diagnostic radiopharmaceuticals with low target:blood ratios. The technique has been used widely in myocardial imaging with iodinated long-chain free fatty acids. Vyska *et al*[257] reported the use of ω-^{123}-I-heptadecanoic acid (IHA) with low uncorrected myocardium:background ratios of 2.01:1 to 2.9:1 in 10 normal human subjects. The correction procedure was based on quantitation of the contribution to the image by the inorganic $^{123}I^-$ pool. The myocardium and an area outside the myocardium e.g. the vena cava were monitored for accumulation of radioactivity after an injection of IHA and again after an injection of ^{123}I-NaI. A proportionality constant for the increase in count rate in the vena cava region and that in the myocardium after the second injection was established. Background subtraction was processed mathematically using the derived constant.

Van der Wall *et al*[258] also reported improved image quality by using a similar background subtraction technique in myocardial scintigraphy with ^{123}I-hexadecanoic and ^{123}I-heptadecanoic acid using the lung, vena cava and aortic root

as the control regions.

Background subtraction of this type is possible because the radioactive species (free iodide) contributing to the background is different from the original imaging agent (iodinated free fatty acids) and because their distributions are also different. The technique is potentially useful in radiobrominated pharmaceuticals imaging studies. Bromide anions are poorly absorbed by most tissues and are eliminated very slowly from the mammalian body thus constituting a steady level of background radioactivity. [^{82}Br]-2'-BrUdR was debrominated extensively in BDF$_1$ mice (Table 9.9 and Fig. 6.5). The percentage of dose retained g^{-1} blood was high from 0.08 hr (9.65%) to 12 hr (8.26%) (Table 9.4). Blood radioactivity after the initial 3 to 4 hr is assumed to be due to the long-lived radiobromide. The tumor showed an increase of radioactivity uptake from 0.08 hr (3.56%) to 12 hr (5.89%) (Table 9.4). However, the maximum tumor:blood ratio recorded was 0.70 at 12 hr. The ratio was much smaller than those for IHA reported by Vyska *et al*, yet the pharmacokinetics of bromide make background subtraction technique of the type described above a potential means of improving ^{82}Br imaging quality.

Another potentially useful background subtraction technique using monoclonal antibody has been reported by Ballou *et al*.[255] ^{131}I-Labelled teratocarcinoma-specific monoclonal antibody and ^{125}I-labelled indifferent antibody were administered and monitored simultaneously. Background contribut-

ion of a non-specific nature as indicated by the ^{123}I radioactivity could then be subtracted from the image projected by the tumor-specific antibody.

The halogenated pyrimidines investigated were of low specific activities because of inherent physical properties of the radionuclides involved. The effect of specific activity on target tissue localization has been reviewed by Counsell and Ice.[4] The authors maintained that the uptake of a radiopharmaceutical by a tissue was determined by the binding affinity and binding capacity of the tissue for the particular radiopharmaceutical. Even if the target tissue or lesion exhibited a high affinity for a given compound selective uptake would not be observed in tracer experiments if the binding capacity was small and the specific activity of the compound was low. This is of particular significance in the case of radiolabelled hormones where binding can be reduced by increasing the relative concentration of the corresponding unlabelled hormones or their analogues. Application of this observation to account for the low uptake of radiohalogenated pyrimdines by Lewis Lung carcinoma will remain speculative until synthesis with no-carrier-added radionuclides of chlorine and bromine is achieved.

The present study has not uncovered any significant diagnostic agents for Lewis Lung tumor. It has, however, demonstrated the potential of halogenated pyrimidines in nuclear medicine especially in conjunction with a proper background subtraction procedure. A nucleoside that is found

inactive in one system does not rule out its potential usefulness in another. It must be evaluated against other target systems. Inactivity in one situation sometimes constitutes selectivity in another. Much knowledge has also been gained about the biological fate of these compounds and their structure-activity relationship. This is invaluable information in future design of new radiopharmaceuticals.

5. Conclusion

1. [^{82}Br]-6-BrU (68.40 MBq mM^{-1}), [^{82}Br]-2'-BrUdR (22.2 MBq mM^{-1}, [^{34}Cl]-3'-ClUdR (5.48 MBq mM^{-1}) and [^{82}Br]-3'-BrUdR (28.99 MBq mM^{-1}) were synthesized. These prepared compounds and [^{34}Cl]-6ClU (5.66 MBq mM^{-1}) were evaluated for preferential tissue uptake in BDF$_1$ mice bearing Lewis Lung carcinomas. No selective tissue uptake was observed for any of the compounds.
2. Excretion of all the compounds tested was mainly renal. Limited biliary excretion was also observed.
3. Dehalogenation was most extensive for [^{82}Br]-2'-BrUdR and least so for [^{34}Cl]-3'-ClUdR.
4. The presence of an electronegative substituent at C-6 of uracil, the lack of a hydrophobic centre and the inability to undergo phosphorylation were probably responsible for the inactivity of the 6-substituted uracils.
5. The lack of observable tumor uptake of [^{82}Br]-2'-BrUdR was probably attributable to its rapid elimination from the body, an *in vivo* debromination process and a change in conformation of the molecule as a result of bromination.
6. The 3'-halogen substituted pyrimidines exhibited no preferential tumor localization. Speculative explanations are the lack of a 3'-OH or oxygen and a contorted conformation as a result of halogenation.

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